PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

B4

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 15/19, C07K 14/54, C12N 15/62, G01N 33/50, C12Q 1/68, A61K 38/19, C12N 5/10, A01K 67/00

A1

(11) International Publication Number: WO 98/28425

(43) International Publication Date: 2 July 1998 (02.07.98)

(21) International Application Number: PCT/US97/22975

(22) International Filing Date: 22 December 1997 (22.12.97)

(30) Priority Data:

60/034,151	23 December 1996 (23.12.96)	US
60/042,222	15 April 1997 (15.04.97)	US

(71) Applicant: SCHERING CORPORATION [US/US]; 2000 Galloping Hill Road, Kenilworth, NJ 07033 (US).

(72) Inventors: BAZAN, J., Fernando; 775 University Drive, Menlo Park, CA 94025 (US). KASTELEIN, Robert, A.; 463 Summit Drive, Redwood City, CA 94062 (US). DE WAAL MALEFYT, Rene; 891 Muender Avenue, Sunnyvale, CA 94040 (US).

(74) Agents: FOULKE, Cynthia, L. et al.; Schering-Plough Corporation, Patent Dept. K-6-1, 1990, 2000 Galloping Hill Road, Kenilworth, NJ 07033-0530 (US).

(81) Designated States: AL, AM, AU, AZ, BA, BB, BG, BR, BY, CA, CN, CZ, EE, GE, GW, HU, ID, IL, IS, JP, KG, KR, KZ, LC, LK, LR, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UZ, VN, YU, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published

With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: MAMMALIAN CYTOKINE RELATED TO IL10

(57) Abstract

Purified genes encoding cytokine from a mammal, reagents related thereto including purified proteins, specific antibodies, and nucleic acids encoding this molecule are provided. Methods of using said reagents and diagnostic kits are also provided.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece		Republic of Macedonia	**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's	**NZ**	New Zealand		
CM	Cameroon		Republic of Korea	**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

MAMMALIAN CYTOKINE RELATED TO IL10

FIELD OF THE INVENTION

The present invention pertains to compositions related to proteins which function in controlling biology and physiology of mammalian cells, e.g., cells of a mammalian immune system. In particular, it provides purified genes, proteins, antibodies, and related reagents useful, e.g., to regulate activation, development, differentiation, and function of various cell types, including hematopoietic cells.

BACKGROUND OF THE INVENTION

Recombinant DNA technology refers generally to the technique of integrating genetic information from a donor source into vectors for subsequent processing, such as through introduction into a host, whereby the transferred genetic information is copied and/or expressed in the new environment. Commonly, the genetic information exists in the form of complementary DNA (cDNA) derived from messenger RNA (mRNA) coding for a desired protein product. The carrier is frequently a plasmid having the capacity to incorporate cDNA for later replication in a host and, in some cases, actually to control expression of the cDNA and thereby direct synthesis of the encoded product in the host.

For some time, it has been known that the mammalian immune response is based on a series of complex cellular interactions, called the "immune network". Recent research has provided new insights into the inner workings of this network. While it remains clear that much of the response does, in fact, revolve around the network-like interactions

of lymphocytes, macrophages, granulocytes, and other cells, immunologists now generally hold the opinion that soluble proteins, known as lymphokines, cytokines, or monokines, play a critical role in controlling these cellular interactions. Thus, there is considerable interest in the isolation, characterization, and mechanisms of action of cell modulatory factors, an understanding of which will lead to significant advancements in the diagnosis and therapy of numerous medical abnormalities, e.g., immune system disorders.

Lymphokines apparently mediate cellular activities in a variety of ways. They have been shown to support the proliferation, growth, and differentiation of pluripotential hematopoietic stem cells into vast numbers of progenitors comprising diverse cellular lineages making up a complex immune system. Proper and balanced interactions between the cellular components are necessary for a healthy immune response. The different cellular lineages often respond in a different manner when lymphokines are administered in conjunction with other agents.

Cell lineages especially important to the immune response include two classes of lymphocytes: B-cells, which can produce and secrete immunoglobulins (proteins with the capability of recognizing and binding to foreign matter to effect its removal), and T-cells of various subsets that secrete lymphokines and induce or suppress the B-cells and various other cells (including other T-cells) making up the immune network. These lymphocytes interact with many other cell types.

Another important cell lineage is the mast cell (which has not been positively identified in all mammalian species), which is a granule-containing connective tissue cell located proximal to capillaries throughout the body. These cells are found in especially high concentrations in the lungs, skin, and gastrointestinal and genitourinary tracts. Mast cells play a central role in allergy-related

disorders, particularly anaphylaxis as follows: when selected antigens crosslink one class of immunoglobulins bound to receptors on the mast cell surface, the mast cell degranulates and releases mediators, e.g., histamine, serotonin, heparin, and prostaglandins, which cause allergic reactions, e.g., anaphylaxis.

Research to better understand and treat various immune disorders has been hampered by the general inability to maintain cells of the immune system in vitro. Immunologists have discovered that culturing these cells can be accomplished through the use of T-cell and other cell supernatants, which contain various growth factors, including many of the lymphokines.

The gene encoding IL-10, originally designated Cytokine Synthesis Inhibitory Factor (CSIF), was isolated in the 1980's. See, e.g., Mosmann, et al., U.S. Patent No. 5,231,012. Since then, much has been learned of the biology and physiology mediated by the cytokine. See, e.g., de Vries and de Waal Malefyt (1995) <u>Interleukin-10</u> Landes Co., Austin, TX.

From the foregoing, it is evident that the discovery and development of new lymphokines, e.g., related to IL-10, could contribute to new therapies for a wide range of degenerative or abnormal conditions which directly or indirectly involve the immune system and/or hematopoietic cells. In particular, the discovery and development of lymphokines which enhance or potentiate the beneficial activities of known lymphokines would be highly advantageous. The present invention provides new interleukin compositions and related compounds, and methods for their use.

SUMMARY OF THE INVENTION

The present invention is directed to mammalian, e.g., rodent, canine, feline, primate, interleukin-BKW (IL-BKW), and its biological activities. It includes nucleic acids

coding for polypeptides themselves and methods for their production and use. The nucleic acids of the invention are characterized, in part, by their homology to cloned complementary DNA (cDNA) sequences enclosed herein, and/or by functional assays for IL-10-like activities applied to the polypeptides, which are typically encoded by these nucleic acids. Methods for modulating or intervening in the control of an immune response are provided.

The present invention is based, in part, upon the discovery of a new cytokine sequence exhibiting high sequence and structural similarity to cellular IL-10. In particular, it provides a gene encoding a protein whose mature size is about 158 amino acids. Functional equivalents exhibiting significant sequence homology will be available from other mammalian, e.g., mouse and rat, and non-mammalian species.

In one embodiment, the invention provides a substantially pure or recombinant soluble IL-BKW, including an antigenic protein or peptide fragment of the soluble IL-BKW. Preferably, the IL-BKW is a full length natural soluble protein from a mammal, including a primate, or may be in a sterile composition. Typically, the soluble IL-BKW lacks the sequence MNFQQRLQSL WTLARPFCPP LLATASQMQM VVLPCLGFTL LLWSQVSG of SEQ ID NO: 2; is a mature polypeptide of SEQ ID NO: 6; or is encoded by a nucleic acid of SEQ ID NO: 5. Alternatively, the soluble IL-BKW is a full length secreted protein which exhibits a post-translational modification pattern distinct from natural soluble IL-BKW.

Functionally, the soluble IL-BKW will typically exhibit an immunological activity functionally antagonistic to IL-10.

The invention also provides a fusion protein comprising sequence of a soluble IL-BKW, but lacking sequence of MNFQQRLQSL WTLARPFCPP LLATASQMQM VVLPCLGFTL LLWSQVSG of SEQ ID NO: 2; is a mature polypeptide of SEQ ID NO: 6; or is encoded by SEQ ID NO: 5.

The invention also provides a method of purifying a soluble IL-BKW protein or peptide from other materials in a mixture comprising contacting said mixture to an antibody to said protein, and separating bound IL-BKW from other materials.

In other embodiments, the invention provides an isolated or recombinant expression vector encoding a soluble IL-BKW. Preferably, the vector encodes a secreted sequence of SEQ ID NO: 2 or 6 and may comprise sequence of SEQ ID NO: 1 or 5.

The invention provides a kit for detection comprising a positive control which is a substantially pure soluble IL-BKW or fragment. The kit also provides a method for detecting in a sample for the presence of a soluble IL-BKW protein, or antibody, comprising testing said sample with the kit.

The invention also provides a method of modulating the physiology of a cell comprising contacting the cell with a substantially pure soluble IL-BKW. In certain embodiments, the cell is a T cell and the modulating of physiology is inactivation of the T cell; or the cell is in a tissue.

The invention also provides a method of making a soluble IL-BKW comprising expressing a vector. The vector may be in a cell, tissue, or organ.

Finally, the invention provides a method of treating an animal having an abnormal immune response by administering to the animal an effective dose of a substantially pure soluble IL-BKW.

BRIEF DESCRIPTION OF THE DRAWING

Figure 1 shows alignment of IL-10 and related cytokines.

DETAILED DESCRIPTION OF THE INVENTION

All references cited herein are incorporated herein by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

I. General

The present invention provides amino acid sequences and DNA sequences encoding various mammalian proteins which are cytokines, e.g., which are secreted molecules which can mediate a signal between immune or other cells. See, e.g., Paul (1994) Fundamental Immunology, Raven Press, N.Y. . The full length cytokines, and fragments, or antagonists will be useful in physiological modulation of cells expressing a receptor. Early data suggests that the cytokine functions to effect the opposite effects that IL-10 does. It is likely that IL-BKW has either stimulatory or inhibitory effects on T-cells, B-cells, natural killer (NK) cells, macrophages, dendritic cells, hematopoietic progenitors, etc. The proteins will also be useful as antigens, e.g., immunogens, for raising antibodies to various epitopes on the protein, both linear and conformational epitopes.

A cDNA encoding IL-BKW was isolated from a human melanoma cell line. The molecule was designated mda7, and was characterized as a novel melanoma differentiation associated gene. SEQ ID NO: 1 and 2. See Jiang, et al. (1995) Oncogene 11:2477-86; Genbank accession number U16261. That paper reported some small homology of the mda7 to human IL-10, but the relevance was unknown. Applicants have analyzed the sequence and believe that the coding region had been misidentified, a fact consistent with the sequence of the mouse gene.

The CDS was proposed by Jiang, *et al.* to run about from 275..895 to encode:

MNFQQRLQSL WTLARPFCPP LLATASQMQM VVLPCLGFTL LLWSQVSGAQ GQEFHFGPCQ
VKGVVPQKLW EAFWAVKDTM QAQDNITSAR LLQQEVLQNV SDAESCYLVH TLLEFYLKTV
FKNYHNRTVE VRTLKSFSTL ANNFVLIVSQ LQPSQENEMF SIRDSAHRRF LLFRRAFKQL
DVEAALTKAL GEVDILLTWM QKFYKL (SEQ ID NO: 2);

however, Applicants propose a mature form of IL-BKW which begins at about the 49th residue, or:

AQGQEFHFGP CQVKGVVPQK LWEAFWAVKD TMQAQDNITS ARLLQQEVLQ NVSDAESCYL VHTLLEFYLK TVFKNYHNRT VEVRTLKSFS TLANNFVLIV SQLQPSQENE MFSIRDSAHR RFLLFRRAFK QLDVEAALTK ALGEVDILLT WMQKFYKL.

In Table 1 various human IL-10 embodiments are compared to human IL-BKW.

TABLE 1

IL-BKW	.MQMVVLPCL	GFTLLLWSQV	SGAQGQEFHF	GPCQVK.GVV	PQKL...WEA
IL-10	MHSSALLCCL	..VLLTGVRA	SPGQGTQSEN	SCTHFP.GNL	PNMLRDLRDA
IL-XX	MLVNFILRCG	..LLLVTLSL	AIAKHKQSSF	TKSCYPRGTL	SQAVDALYIK
IL-BKW	FWAVKDTMQA	QDNITSAR.L	LQQEVLQNVS	DAESCYLVHT	LLEFYLKTVF
IL-10	FSRVKTFFQM	KDQLDNL..L	LKESLLEDFK	GYLGCQALSE	MIQFYLEEVM
IL-XX	AAWLKATIP.	EDRIKNIR.L	LKKKTKKQFM	K..NCQFQEQ	LLSFFNEDVF
IL-BKW	KNYHNRTVEV	RTLKSFSTLA	NNFVLIVSQL	QPSQENEMFS	IRDSAHRRFL
IL-10	PQAENQDPDI	..KAHVNSLG	ENLKTLRLRL	RRCHR...FL	PCENKSKAVE
IL-XX	GQLQLQG...	...CKKIRFV	EDFHTLRQKL	SHCIS...CA	SSAREMKSIT
IL-BKW	LFRRAFKQLD	VEAALTKALG	EVDILLTWMQ	KFYKL....	
IL-10	QVKNAFNKLQ	.EKGIYKAMS	EFDIFINYIE	AYMTMKIRN	
IL-XX	RMKRIFYRIG	.NKGIYKAIS	ELDILLSWIK	KLLESSQ..	

hIL-BKW (SEQ ID NO: 2)
hIL-10 (SEQ ID NO: 3)
hIL-XX (SEQ ID NO: 4)
See Knappe, et al., USSN 08/718,753.

A murine clone for IL-BKW (mIL-BKW) has also been isolated from an activated mouse thymocyte cDNA library (A. Zlotnik, DNAX Res. Institute, Palo Alto, CA). SEQ ID NO: 5 and 6. The signal sequence runs from about Met1 to Gly23. The mature polypeptide begins at about Leu24. The conserved D-helix, implicated in receptor binding, runs from about Glu158 to Leu181. There is no corresponding ATG upstream of the leader sequence as found in the human, possibly indicating that this molecule is membrane bound.

mIL-BKW possess a high degree of amino acid sequence identity to the human counterpart. An alignment between hIL-10, mIL-10, hIL-BKW, and mIL-BKW reveals that the D-helix is highly conserved. See Table 2 and SEQ ID NO: 2, 3, 6, and 7.

Table 2 compares human and mouse IL-10 and human and mouse IL-BKW. In IL-10, this helix seems particularly important in receptor binding. Therefore, IL-BKW and IL-10 may share the same receptor subunit.

TABLE 2

hIL10	..MHSSALLC	C.LVLLTGVR	AS..PGQGTQ	SENSCTHFPG	NLPNMLRDLR
mIL10	..MPGSALLC	C.LLLLTGMR	IS..RGQYSR	EDNNCTHFPV	GQSHMLLELR
hILBKW	..MQMVVLPC	LGFTLLLWSQ	VSGAQGQEFH	F.GPCQVKGV	....VPQKLW
mILBKW	MSWGLQILPC	LSLILLLWNQ	VPGLEGQEFR	S.GSCQVTGV	....VLPELW
hIL10	DAFSRVKTFF	QMKDQLDNL.	LLKESLLEDF	KGYLGCQALS	EMIQFYLEEV
mIL10	TAFSQVKTFF	QTKDQLDNI.	LLTDSLMQDF	KGYLGCQALS	EMIQFYLVEV
hILBKW	EAFWAVKDTM	QAQDNITSAR	LLQQEVLQNV	SDAESCYLVH	TLLEFYLKTV
mILBKW	EAFWTVKNTV	QTQDDITSIR	LLKPQVLRNV	SGAESCYLAH	SLLKFYLNTV
hIL10	MPQAENQDPD	IK..AHVNSL	GENLKTLRLR	LRR...CHRF	LPCENKSKAV
mIL10	MPQAEKHGPE	IK..EHLNSL	GEKLKTLRMR	LRR...CHRF	LPCENKSKAV
hILBKW	FKNYHNRTVE	VRTLKSFSTL	ANNFVLIVSQ	LQPSQENEMF	SIRDSAHRRF
mILBKW	FKNYHSKIAK	FKVLRSFSTL	ANNFIVIMSQ	LQPSKDNSML	PISESAHQRF
hIL10	EQVKNAFNKL	Q. EKGIYKAM	SEFDIFINYI	EAYMTMKIR	N
mIL10	EQVKSDFNKL	Q. DQGVYKAM	NEFDIFINCI	EAYMMIKMK	S
hILBKW	LLFRRAFKQL	DV EAALTKAL	GEVDILLTWM	QKFYKL...	
mILBKW	LLFRRAFKQL	DT EVALVKAF	GEVDILLTWM	QKFYHL...	
			D-helix		

hIL-10 (SEQ ID NO:3)
mIL-10 (SEQ ID NO: 7)
hIL-BKW (SEQ ID NO: 2)
mIL-BKW (SEQ ID NO: 6).

Chromosomal mapping using mIL-BKW as a probe revealed that this cytokine maps to the central region of mouse chromosome 1 in between the regions of homology for human chromosomes 2q and 1q. Interestingly, mIL-BKW is located directly adjacent to the gene for mIL-10. Defects in this particular locus have been implicated in immune disorders, e.g., dominant hemimelia. See, e.g., Carter (1954) <u>Mouse News Lett.</u> 11:16; Higgins, et al. (1992) <u>Genet. Res.</u> 60:53-60; and Machado, et al. (1976) <u>Am. J. Pathol.</u> 85:515-518.

The protein had been characterized as an antigen with a predicted hydrophobic stretch encompassing residues 25-45 of the protein. But experiments failed to confirm that a membrane form exists. Applicants believe that the predicted hydrophobic stretch is actually a signal sequence which is removed from the mature protein, which is likely to start at

either residue 47 (Ser) or 49 (Ala), as designated in the original publication.

Applicants believe that the gene encodes a small soluble cytokine-like protein, of about 158 amino acids. The pre-sequence probably starts at either the M at position 28 or 30, thus providing an N-terminal signal sequence of about 17-21 amino acids. See Table 1 and SEQ. ID. NO: 1 and 2. IL-BKW exhibits structural motifs characteristic of a member of the short chain cytokines. Compare, e.g., IL-BKW, cellular IL-10s from mouse and human, EBV viral IL-10, and the Equine herpesvirus IL-10, all sequences available from GenBank. See Table 1.

The structural homology of IL-BKW to the related IL-10 proteins suggests related function of this molecule. IL-BKW is a small chain cytokine. Early experiments suggest that the new cytokine likely mediates immune functions via a receptor of the class of cytokine receptors, though it seems not to share all parts of a functional IL-10 receptor complex.

IL-BKW agonists, or antagonists, may also act as functional or receptor antagonists, e.g., which block IL-10 binding to its receptor, or mediating the opposite actions. Thus, IL-BKW, or its antagonists, may be useful in the treatment of abnormal immune disorders, e.g., T cell immune deficiencies, chronic inflammation, or tissue rejection.

The natural antigens are capable of mediating various biochemical responses which lead to biological or physiological responses in target cells. The embodiments characterized herein are from human and mouse, but other primate, or other species counterparts are expected to exist in nature. Additional sequences for proteins in other mammalian species, e.g., primates, canines, felines, and rodents, should also be available. See below. The descriptions below are directed, for exemplary purposes, to a human IL-BKW, but are likewise applicable to related embodiments from other species.

The human and mouse IL-BKW proteins exhibit structural features characteristic of short chain cytokines.

II. Purified IL-BKW

Human IL-BKW amino acid sequence, lacking the N-terminal sequence, e.g., MNFQQRLQSL WTLARPFCPP LLATASQMQM VVLPCLGFTL LLWSQVSG, is shown as one embodiment within SEQ ID NO: 2. The corresponding mIL-BKW amino acid sequence is shown in SEQ ID NO: 6. These amino acid sequences, provided amino to carboxy, are important in providing sequence information in the cytokine allowing for distinguishing the protein antigen from other proteins and exemplifying numerous variants. Moreover, the peptide sequences allow preparation of peptides to generate antibodies to recognize such segments, and nucleotide sequences allow preparation of oligonucleotide probes, both of which are strategies for detection or isolation, e.g., cloning, of genes encoding such sequences. Potential glycosylation sites on the human sequence are asn31-thr39 and asn51-ser53; and on the mouse asn51-ser53.

As used herein, the term "human soluble IL-BKW" shall encompass, when used in a protein context, a protein having amino acid sequence corresponding to a soluble polypeptide shown in SEQ ID NO: 2, e.g., lacking the amino terminal portion described as membrane associated (MNFQQRLQSL WTLARPFCPP LLATASQMQM VVLPCLGFTL LLWSQVSG). Such will lack the first 45 residues described as the N terminus by Jiang, et al., or significant fragments thereof. "Mouse IL-BKW" shall encompass the amino acid sequence corresponding to SEQ ID NO: 6. Binding components, e.g., antibodies, typically bind to an IL-BKW with high affinity, e.g., at least about 100 nM, usually better than about 30 nM, preferably better than about 10 nM, and more preferably at better than about 3 nM. Homologous proteins would be found in mammalian species other than human, e.g., other primates or rodents. Non-mammalian species should also possess structurally or

functionally related genes and proteins, e.g., birds or amphibians.

The term "polypeptide" as used herein includes a significant fragment or segment, and encompasses a stretch of amino acid residues of at least about 8 amino acids, generally at least about 12 amino acids, typically at least about 16 amino acids, preferably at least about 20 amino acids, and, in particularly preferred embodiments, at least about 30 or more amino acids, e.g., 35, 40, 45, 50, etc. Such fragments may have ends which begin and/or end at virtually all positions, e.g., beginning at residues 1, 2, 3, etc., and ending at, e.g., 150, 149, 148, etc., in all combinations. Particularly interesting peptides have ends corresponding to structural domain boundaries, e.g., helices A, B, C, and/or D. See Table 1, Table 2, and Figure 1. Note that the sequence of IL-BKW exhibits particular identity to cellular IL-10 in the region from residue 126-137, and the other regions exhibit greater extents of IL-BKW specific sequence.

The term "binding composition" refers to molecules that bind with specificity to IL-BKW, e.g., in an antibody-antigen interaction. It also includes compounds, e.g., proteins, which specifically associate with IL-BKW, including in a natural physiologically relevant protein-protein interaction, either covalent or non-covalent. The molecule may be a polymer, or chemical reagent. A functional analog may be a protein with structural modifications, or it may be a molecule which has a molecular shape which interacts with the appropriate binding determinants. The compounds may serve as agonists or antagonists of a receptor binding interaction, see, e.g., Goodman, et al. (eds.) (1990) Goodman & Gilman's: The Pharmacological Bases of Therapeutics (8th ed.), Pergamon Press.

Substantially pure typically means that the protein is free from other contaminating proteins, nucleic acids, or

other biologicals derived from the original source organism. Purity may be assayed by standard methods, typically by weight, and will ordinarily be at least about 40% pure, generally at least about 50% pure, often at least about 60% pure, typically at least about 80% pure, preferably at least about 90% pure, and in most preferred embodiments, at least about 95% pure. Carriers or excipients will often be added.

Solubility of a polypeptide or fragment depends upon the environment and the polypeptide. Many parameters affect polypeptide solubility, including temperature, electrolyte environment, size and molecular characteristics of the polypeptide, and nature of the solvent. Typically, the temperature at which the polypeptide is used ranges from about 4° C to about 65° C. Usually the temperature at use is greater than about 18° C. For diagnostic purposes, the temperature will usually be about room temperature or warmer, but less than the denaturation temperature of components in the assay. For therapeutic purposes, the temperature will usually be body temperature, typically about 37° C for humans and mice, though under certain situations the temperature may be raised or lowered in situ or in vitro.

The size and structure of the polypeptide should generally be in a substantially stable state, and usually not in a denatured state. The polypeptide may be associated with other polypeptides in a quaternary structure, e.g., to confer solubility, or associated with lipids or detergents.

The solvent and electrolytes will usually be a biologically compatible buffer, of a type used for preservation of biological activities, and will usually approximate a physiological aqueous solvent. Usually the solvent will have a neutral pH, typically between about 5 and 10, and preferably about 7.5. On some occasions, one or more detergents will be added, typically a mild non-denaturing one, e.g., CHS (cholesteryl hemisuccinate) or CHAPS (3-[3-cholamidopropyl)dimethylammonio]-1-propane

sulfonate), or a low enough concentration as to avoid significant disruption of structural or physiological properties of the protein.

III. Physical Variants

This invention also encompasses proteins or peptides having substantial amino acid sequence identity with the amino acid sequence of the IL-BKW antigen. The variants include species, polymorphic, or allelic variants.

Amino acid sequence homology, or sequence identity, is determined by optimizing residue matches, if necessary, by introducing gaps as required. See also Needleham, et al. (1970) J. Mol. Biol. 48:443-453; Sankoff, et al. (1983) Chapter One in Time Warps, String Edits, and Macromolecules: The Theory and Practice of Sequence Comparison, Addison-Wesley, Reading, MA; and software packages from IntelliGenetics, Mountain View, CA; and the University of Wisconsin Genetics Computer Group, Madison, WI. Sequence identity changes when considering conservative substitutions as matches. Conservative substitutions typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid; asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine. The conservation may apply to biological features, functional features, or structural features. Homologous amino acid sequences are typically intended to include natural polymorphic or allelic and interspecies variations in each respective protein sequence. Typical homologous proteins or peptides will have from 25-100% identity (if gaps can be introduced), to 50-100% identity (if conservative substitutions are included) with the amino acid sequence of the IL-BKW. Identity measures will be at least about 35%, generally at least about 40%, often at least about 50%, typically at least about 60%, usually at least about 70%, preferably at least about 80%, and more preferably at least about 90%.

The isolated IL-BKW DNA can be readily modified by nucleotide substitutions, nucleotide deletions, nucleotide insertions, and inversions of nucleotide stretches. These modifications result in novel DNA sequences which encode these antigens, their derivatives, or proteins having similar physiological, immunogenic, antigenic, or other functional activity. These modified sequences can be used to produce mutant antigens or to enhance expression. Enhanced expression may involve gene amplification, increased transcription, increased translation, and other mechanisms. "Mutant IL-BKW" encompasses a polypeptide otherwise falling within the sequence identity definition of the IL-BKW as set forth above, but having an amino acid sequence which differs from that of IL-BKW as normally found in nature, whether by way of deletion, substitution, or insertion. This generally includes proteins having significant identity with a protein having sequence of SEQ ID NO: 2 or 6, and as sharing various biological activities, e.g., antigenic or immunogenic, with those sequences, and in preferred embodiments contain most of the full length disclosed sequences. Full length sequences will typically be preferred, though truncated versions will also be useful, likewise, genes or proteins found from natural sources are typically most desired. Similar concepts apply to different IL-BKW proteins, particularly those found in various warm blooded animals, e.g., mammals and birds. These descriptions are generally meant to encompass all IL-BKW proteins, not limited to the particular mouse embodiments specifically discussed.

IL-BKW mutagenesis can also be conducted by making amino acid insertions or deletions. Substitutions, deletions, insertions, or any combinations may be generated to arrive at a final construct. Insertions include amino- or carboxy- terminal fusions. Random mutagenesis can be conducted at a target codon and the expressed mutants can then be screened for the desired activity. Methods for

making substitution mutations at predetermined sites in DNA having a known sequence are well known in the art, e.g., by M13 primer mutagenesis or polymerase chain reaction (PCR) techniques. See, e.g., Sambrook, et al. (1989); Ausubel, et al. (1987 and Supplements); and Kunkel, et al. (1987) Methods in Enzymol. 154:367-382.

The present invention also provides recombinant proteins, e.g., heterologous fusion proteins using segments from these proteins. A heterologous fusion protein is a fusion of proteins or segments which are naturally not normally fused in the same manner. A similar concept applies to heterologous nucleic acid sequences.

In addition, new constructs may be made from combining similar functional domains from other proteins. For example, target-binding or other segments may be "swapped" between different new fusion polypeptides or fragments. See, e.g., Cunningham, et al. (1989) Science 243:1330-1336; and O'Dowd, et al. (1988) J. Biol. Chem. 263:15985-15992.

The phosphoramidite method described by Beaucage and Carruthers (1981) Tetra. Letts. 22:1859-1862, will produce suitable synthetic DNA fragments. A double stranded fragment will often be obtained either by synthesizing the complementary strand and annealing the strand together under appropriate conditions or by adding the complementary strand using DNA polymerase with an appropriate primer sequence, e.g., PCR techniques.

IV. Functional Variants

The blocking of physiological response to IL-BKWs may result from the competitive inhibition of binding of the ligand to its receptor. Preliminary results suggest that the IL-BKW does not bind to the same as the described subunit of the receptor for IL-10. An IL-BKW antagonist would be expected to have the opposite effect as IL-BKW.

In vitro assays of the present invention will often use isolated protein, soluble fragments comprising receptor binding segments of these proteins, or fragments attached to

solid phase substrates. These assays will also allow for the diagnostic determination of the effects of either binding segment mutations and modifications, or cytokine mutations and modifications, e.g., IL-BKW analogs.

This invention also contemplates the use of competitive drug screening assays, e.g., where neutralizing antibodies to the cytokine, or receptor binding fragments compete with a test compound.

"Derivatives" of IL-BKW antigens include amino acid sequence mutants from naturally occurring forms, glycosylation variants, and covalent or aggregate conjugates with other chemical moieties. Covalent derivatives can be prepared by linkage of functionalities to groups which are found in IL-BKW amino acid side chains or at the N- or C-termini, e.g., by standard means. See, e.g., Lundblad and Noyes (1988) Chemical Reagents for Protein Modification, vols. 1-2, CRC Press, Inc., Boca Raton, FL; Hugli (ed.) (1989) Techniques in Protein Chemistry, Academic Press, San Diego, CA; and Wong (1991) Chemistry of Protein Conjugation and Cross Linking, CRC Press, Boca Raton, FL.

In particular, glycosylation alterations are included, e.g., made by modifying the glycosylation patterns of a polypeptide during its synthesis and processing, or in further processing steps. See, e.g., Elbein (1987) Ann. Rev. Biochem. 56:497-534. Also embraced are versions of the peptides with the same primary amino acid sequence which have other minor modifications, including phosphorylated amino acid residues, e.g., phosphotyrosine, phosphoserine, or phosphothreonine.

Fusion polypeptides between IL-BKWs and other homologous or heterologous proteins are also provided. Many cytokine receptors or other surface proteins are multimeric, e.g., homodimeric entities, and a repeat construct may have various advantages, including lessened susceptibility to proteolytic cleavage. Typical examples are fusions of a reporter polypeptide, e.g., luciferase, with a segment or

domain of a protein, e.g., a receptor-binding segment, so that the presence or location of the fused ligand may be easily determined. See, e.g., Dull, et al., U.S. Patent No. 4,859,609. Other gene fusion partners include bacterial ß-galactosidase, trpE, Protein A, ß-lactamase, alpha amylase, alcohol dehydrogenase, yeast alpha mating factor, and detection or purification tags such as a FLAG sequence of His6 sequence. See, e.g., Godowski, et al. (1988) Science 241:812-816.

Fusion peptides will typically be made by either recombinant nucleic acid methods or by synthetic polypeptide methods. Techniques for nucleic acid manipulation and expression are described generally, e.g., in Sambrook, et al. (1989) Molecular Cloning: A Laboratory Manual (2d ed.), vols. 1-3, Cold Spring Harbor Laboratory; and Ausubel, et al. (eds.) (1993) Current Protocols in Molecular Biology, Greene and Wiley, NY. Techniques for synthesis of polypeptides are described, e.g., in Merrifield (1963) J. Amer. Chem. Soc. 85:2149-2156; Merrifield (1986) Science 232: 341-347; Atherton, et al. (1989) Solid Phase Peptide Synthesis: A Practical Approach, IRL Press, Oxford; and Grant (1992) Synthetic Peptides: A User's Guide, W.H. Freeman, NY. Refolding methods may be applicable to synthetic proteins.

This invention also contemplates the use of derivatives of IL-BKW proteins other than variations in amino acid sequence or glycosylation. Such derivatives may involve covalent or aggregative association with chemical moieties or protein carriers. Covalent or aggregative derivatives will be useful as immunogens, as reagents in immunoassays, or in purification methods such as for affinity purification of binding partners, e.g., other antigens. An IL-BKW can be immobilized by covalent bonding to a solid support such as cyanogen bromide-activated SEPHAROSE, by methods which are well known in the art, or adsorbed onto polyolefin surfaces, with or without glutaraldehyde cross-linking, for use in the

assay or purification of anti-IL-BKW antibodies or an alternative binding composition. The IL-BKW proteins can also be labeled with a detectable group, e.g., for use in diagnostic assays. Purification of IL-BKW may be effected by an immobilized antibody or complementary binding partner, e.g., binding portion of a receptor.

A solubilized IL-BKW or fragment of this invention can be used as an immunogen for the production of antisera or antibodies specific for binding. Purified antigen can be used to screen monoclonal antibodies or antigen-binding fragments, encompassing antigen binding fragments of natural antibodies, e.g., Fab, Fab', $F(ab)_2$, etc. Purified IL-BKW antigens can also be used as a reagent to detect antibodies generated in response to the presence of elevated levels of the cytokine, which may be diagnostic of an abnormal or specific physiological or disease condition. This invention contemplates antibodies raised against amino acid sequences encoded by nucleotide sequence shown in SEQ ID NO: 1 of 5, or fragments of proteins containing it. In particular, this invention contemplates antibodies having binding affinity to or being raised against specific domains, e.g., helices A, B, C, or D.

The present invention contemplates the isolation of additional closely related species variants. Southern and Northern blot analysis will establish that similar genetic entities exist in other mammals. It is likely that IL-BKWs are widespread in species variants, e.g., rodents, lagomorphs, carnivores, artiodactyla, perissodactyla, and primates.

The invention also provides means to isolate a group of related antigens displaying both distinctness and similarities in structure, expression, and function. Elucidation of many of the physiological effects of the molecules will be greatly accelerated by the isolation and characterization of additional distinct species or polymorphic variants of them. In particular, the present

invention provides useful probes for identifying additional homologous genetic entities in different species.

The isolated genes will allow transformation of cells lacking expression of an IL-BKW, e.g., either species types or cells which lack corresponding proteins and exhibit negative background activity. This should allow analysis of the function of IL-BKW in comparison to untransformed control cells.

Dissection of critical structural elements which effect the various physiological functions mediated through these antigens is possible using standard techniques of modern molecular biology, particularly in comparing members of the related class. See, e.g., the homolog-scanning mutagenesis technique described in Cunningham, et al. (1989) Science 243:1339-1336; and approaches used in O'Dowd, et al. (1988) J. Biol. Chem. 263:15985-15992; and Lechleiter, et al. (1990) EMBO J. 9:4381-4390.

Intracellular functions would probably involve receptor signaling. However, protein internalization may occur under certain circumstances, and interaction between intracellular components and cytokine may occur. Specific segments of interaction of IL-BKW with interacting components may be identified by mutagenesis or direct biochemical means, e.g., cross-linking or affinity methods. Structural analysis by crystallographic or other physical methods will also be applicable. Further investigation of the mechanism of signal transduction will include study of associated components which may be isolatable by affinity methods or by genetic means, e.g., complementation analysis of mutants.

Further study of the expression and control of IL-BKW will be pursued. The controlling elements associated with the antigens should exhibit differential physiological, developmental, tissue specific, or other expression patterns. Upstream or downstream genetic regions, e.g., control elements, are of interest.

Structural studies of the IL-BKW antigens will lead to design of new antigens, particularly analogs exhibiting agonist or antagonist properties on the molecule. This can be combined with previously described screening methods to isolate antigens exhibiting desired spectra of activities.

V. Antibodies

Antibodies can be raised to various epitopes of the IL-BKW proteins, including species, polymorphic, or allelic variants, and fragments thereof, both in their naturally occurring forms and in their recombinant forms. Additionally, antibodies can be raised to IL-BKWs in either their active forms or in their inactive forms, including native or denatured versions. Anti-idiotypic antibodies are also contemplated.

Antibodies, including binding fragments and single chain versions, against predetermined fragments of the antigens can be raised by immunization of animals with conjugates of the fragments with immunogenic proteins. Monoclonal antibodies are prepared from cells secreting the desired antibody. These antibodies can be screened for binding to normal or defective IL-BKWs, or screened for agonistic or antagonistic activity, e.g., mediated through a receptor. Antibodies may be agonistic or antagonistic, e.g., by sterically blocking binding to a receptor. These monoclonal antibodies will usually bind with at least a K_D of about 1 mM, more usually at least about 300 µM, typically at least about 100 µM, more typically at least about 30 µM, preferably at least about 10 µM, and more preferably at least about 3 µM or better.

The antibodies of this invention can also be useful in diagnostic applications. As capture or non-neutralizing antibodies, they can be screened for ability to bind to the antigens without inhibiting binding to a receptor. As neutralizing antibodies, they can be useful in competitive binding assays. They will also be useful in detecting or quantifying IL-BKW protein or its receptors. See, e.g.,

Chan (ed.) (1987) Immunology: A Practical Guide, Academic Press, Orlando, FLA; Price and Newman (eds.) (1991) Principles and Practice of Immunoassay, Stockton Press, N.Y.; and Ngo (ed.) (1988) Nonisotopic Immunoassay, Plenum Press, N.Y. Cross absorptions or other tests will identify antibodies which exhibit various spectra of specificities, e.g., unique or shared species specificities.

Further, the antibodies, including antigen binding fragments, of this invention can be potent antagonists that bind to the antigen and inhibit functional binding, e.g., to a receptor which may elicit a biological response. They also can be useful as non-neutralizing antibodies and can be coupled to toxins or radionuclides so that when the antibody binds to antigen, a cell expressing it, e.g., on its surface, is killed. Further, these antibodies can be conjugated to drugs or other therapeutic agents, either directly or indirectly by means of a linker, and may effect drug targeting.

Antigen fragments may be joined to other materials, particularly polypeptides, as fused or covalently joined polypeptides to be used as immunogens. An antigen and its fragments may be fused or covalently linked to a variety of immunogens, such as keyhole limpet hemocyanin, bovine serum albumin, tetanus toxoid, etc. See Microbiology, Hoeber Medical Division, Harper and Row, 1969; Landsteiner (1962) Specificity of Serological Reactions, Dover Publications, New York; Williams, et al. (1967) Methods in Immunology and Immunochemistry, vol. 1, Academic Press, New York; and Harlow and Lane (1988) Antibodies: A Laboratory Manual, CSH Press, NY, for descriptions of methods of preparing polyclonal antisera.

In some instances, it is desirable to prepare monoclonal antibodies from various mammalian hosts, such as mice, rodents, primates, humans, etc. Description of techniques for preparing such monoclonal antibodies may be found in, e.g., Stites, et al. (eds.) Basic and Clinical

Immunology (4th ed.), Lange Medical Publications, Los Altos, CA, and references cited therein; Harlow and Lane (1988) Antibodies: A Laboratory Manual, CSH Press; Goding (1986) Monoclonal Antibodies: Principles and Practice (2d ed.), Academic Press, New York; and particularly in Kohler and Milstein (1975) in Nature 256:495-497, which discusses one method of generating monoclonal antibodies.

Other suitable techniques involve in vitro exposure of lymphocytes to the antigenic polypeptides or alternatively to selection of libraries of antibodies in phage or similar vectors. See, Huse, et al. (1989) "Generation of a Large Combinatorial Library of the Immunoglobulin Repertoire in Phage Lambda," Science 246:1275-1281; and Ward, et al. (1989) Nature 341:544-546. The polypeptides and antibodies of the present invention may be used with or without modification, including chimeric or humanized antibodies. Frequently, the polypeptides and antibodies will be labeled by joining, either covalently or non-covalently, a substance which provides for a detectable signal. A wide variety of labels and conjugation techniques are known and are reported extensively in both the scientific and patent literature. Suitable labels include radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent moieties, chemiluminescent moieties, magnetic particles, and the like. Patents, teaching the use of such labels include U.S. Patent Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241. Also, recombinant immunoglobulins may be produced, see Cabilly, U.S. Patent No. 4,816,567; Moore, et al., U.S. Patent No. 4,642,334; and Queen, et al. (1989) Proc. Nat'l Acad. Sci. USA 86:10029-10033.

The antibodies of this invention can also be used for affinity chromatography in isolating the protein. Columns can be prepared where the antibodies are linked to a solid support. See, e.g., Wilchek et al. (1984) Meth. Enzymol. 104:3-55.

Antibodies raised against each IL-BKW will also be useful to raise anti-idiotypic antibodies. These will be useful in detecting or diagnosing various immunological conditions related to expression of the respective antigens.

VI. Nucleic Acids

The described peptide sequences and the related reagents are useful in detecting, isolating, or identifying a DNA clone encoding IL-BKW, e.g., from a natural source. Typically, it will be useful in isolating a gene from mammal, and similar procedures will be applied to isolate genes from other species, e.g., warm blooded animals, such as birds and mammals. Cross hybridization will allow isolation of IL-BKW from the same, e.g., polymorphic variants, or other species. A number of different approaches should be available to successfully isolate a suitable nucleic acid clone.

The purified protein or defined peptides are useful for generating antibodies by standard methods, as described above. Synthetic peptides or purified protein can be presented to an immune system to generate monoclonal or polyclonal antibodies. See, e.g., Coligan (1991) Current Protocols in Immunology Wiley/Greene; and Harlow and Lane (1989) Antibodies: A Laboratory Manual, Cold Spring Harbor Press.

For example, the specific binding composition could be used for screening of an expression library made from a cell line which expresses an IL-BKW. Screening of intracellular expression can be performed by various staining or immunofluorescence procedures. Binding compositions could be used to affinity purify or sort out cells expressing a surface fusion protein.

The peptide segments can also be used to predict appropriate oligonucleotides to screen a library. The genetic code can be used to select appropriate oligonucleotides useful as probes for screening. See, e.g., SEQ ID NO: 1 or 5. In combination with polymerase chain

reaction (PCR) techniques, synthetic oligonucleotides will be useful in selecting correct clones from a library. Complementary sequences will also be used as probes, primers, or antisense strands. Various fragments should be particularly useful, e.g., coupled with anchored vector or poly-A complementary PCR techniques or with complementary DNA of other peptides.

This invention contemplates use of isolated DNA or fragments to encode a biologically active corresponding IL-BKW polypeptide, particularly lacking the portion coding the untranslated 5' portion of the described sequence. In addition, this invention covers isolated or recombinant DNA which encodes a biologically active protein or polypeptide and which is capable of hybridizing under appropriate conditions with the DNA sequences described herein. Said biologically active protein or polypeptide can be an intact antigen, or fragment, and have an amino acid sequence disclosed in, e.g., SEQ ID NO: 2 or 6, particularly a mature peptide of SEQ ID NO: 6, or secreted mature polypeptide of SEQ ID NO: 2. Further, this invention covers the use of isolated or recombinant DNA, or fragments thereof, which encode proteins which exhibit high identity to a secreted IL-BKW. The isolated DNA can have the respective regulatory sequences in the 5' and 3' flanks, e.g., promoters, enhancers, poly-A addition signals, and others.

An "isolated" nucleic acid is a nucleic acid, e.g., an RNA, DNA, or a mixed polymer, which is substantially separated from other components which naturally accompany a native sequence, e.g., ribosomes, polymerases, and/or flanking genomic sequences from the originating species. The term embraces a nucleic acid sequence which has been removed from its naturally occurring environment, and includes recombinant or cloned DNA isolates and chemically synthesized analogs or analogs biologically synthesized by heterologous systems. A substantially pure molecule includes isolated forms of the molecule. Generally, the

nucleic acid will be in a vector or fragment less than about 50 kb, usually less than about 30 kb, typically less than about 10 kb, and preferably less than about 6 kb.

An isolated nucleic acid will generally be a homogeneous composition of molecules, but will, in some embodiments, contain minor heterogeneity. This heterogeneity is typically found at the polymer ends or portions not critical to a desired biological function or activity.

A "recombinant" nucleic acid is defined either by its method of production or its structure. In reference to its method of production, e.g., a product made by a process, the process is use of recombinant nucleic acid techniques, e.g., involving human intervention in the nucleotide sequence, typically selection or production. Alternatively, it can be a nucleic acid made by generating a sequence comprising fusion of two fragments which are not naturally contiguous to each other, but is meant to exclude products of nature, e.g., naturally occurring mutants. Thus, e.g., products made by transforming cells with any unnaturally occurring vector is encompassed, as are nucleic acids comprising sequence derived using any synthetic oligonucleotide process. Such is often done to replace a codon with a redundant codon encoding the same or a conservative amino acid, while typically introducing or removing a sequence recognition site.

Alternatively, it is performed to join together nucleic acid segments of desired functions to generate a single genetic entity comprising a desired combination of functions not found in the commonly available natural forms. Restriction enzyme recognition sites are often the target of such artificial manipulations, but other site specific targets, e.g., promoters, DNA replication sites, regulation sequences, control sequences, or other useful features may be incorporated by design. A similar concept is intended for a recombinant, e.g., fusion, polypeptide. Specifically

included are synthetic nucleic acids which, by genetic code redundancy, encode polypeptides similar to fragments of these antigens, and fusions of sequences from various different species or polymorphic variants.

A significant "fragment" in a nucleic acid context is a contiguous segment of at least about 17 nucleotides, generally at least about 22 nucleotides, ordinarily at least about 29 nucleotides, more often at least about 35 nucleotides, typically at least about 41 nucleotides, usually at least about 47 nucleotides, preferably at least about 55 nucleotides, and in particularly preferred embodiments will be at least about 60 or more nucleotides, e.g., 67, 73, 81, 89, 95, etc.

A DNA which codes for an IL-BKW protein will be particularly useful to identify genes, mRNA, and cDNA species which code for related or similar proteins, as well as DNAs which code for homologous proteins from different species. There are likely homologs in other species, including primates, rodents, canines, felines, and birds. Various IL-BKW proteins should be homologous and are encompassed herein. However, even proteins that have a more distant evolutionary relationship to the antigen can readily be isolated under appropriate conditions using these sequences if they are sufficiently homologous. Primate IL-BKW proteins are of particular interest.

Recombinant clones derived from the genomic sequences, e.g., containing introns, will be useful for transgenic studies, including, e.g., transgenic cells and organisms, and for gene therapy. See, e.g., Goodnow (1992) "Transgenic Animals" in Roitt (ed.) Encyclopedia of Immunology, Academic Press, San Diego, pp. 1502-1504; Travis (1992) Science 256:1392-1394; Kuhn, et al. (1991) Science 254:707-710; Capecchi (1989) Science 244:1288; Robertson (1987)(ed.) Teratocarcinomas and Embryonic Stem Cells: A Practical Approach, IRL Press, Oxford; and Rosenberg (1992) J. Clinical Oncology 10:180-199.

Substantial homology, e.g., identity, in the nucleic acid sequence comparison context means either that the segments, or their complementary strands, when compared, are identical when optimally aligned, with appropriate nucleotide insertions or deletions, in at least about 50% of the nucleotides, generally at least about 58%, ordinarily at least about 65%, often at least about 71%, typically at least about 77%, usually at least about 85%, preferably at least about 95 to 98% or more, and in particular embodiments, as high as about 99% or more of the nucleotides. Alternatively, substantial homology exists when the segments will hybridize under selective hybridization conditions, to a strand, or its complement, typically using a sequence of IL-BKW, e.g., in SEQ ID NO: 1, or 5. Typically, selective hybridization will occur when there is at least about 55% identity over a stretch of at least about 30 nucleotides, preferably at least about 75% over a stretch of about 25 nucleotides, and most preferably at least about 90% over about 20 nucleotides. See, Kanehisa (1984) Nuc. Acids Res. 12:203-213. The length of identity comparison, as described, may be over longer stretches, and in certain embodiments will be over a stretch of at least about 17 nucleotides, usually at least about 28 nucleotides, typically at least about 40 nucleotides, and preferably at least about 75 to 100 or more nucleotides.

Stringent conditions, in referring to homology in the hybridization context, will be stringent combined conditions of salt, temperature, organic solvents, and other parameters, typically those controlled in hybridization reactions. Stringent temperature conditions will usually include temperatures in excess of about 30° C, usually in excess of about 37° C, typically in excess of about 55° C, preferably in excess of about 70° C. Stringent salt conditions will ordinarily be less than about 1000 mM, usually less than about 400 mM, typically less than about 250 mM, preferably less than about 150 mM, including about

100, 50, or even 20 mM. However, the combination of parameters is much more important than the measure of any single parameter. See, e.g., Wetmur and Davidson (1968) J. Mol. Biol. 31:349-370.

IL-BKW from other mammalian species can be cloned and isolated by cross-species hybridization of closely related species. Homology may be relatively low between distantly related species, and thus hybridization of relatively closely related species is advisable. Alternatively, preparation of an antibody preparation which exhibits less species specificity may be useful in expression cloning approaches.

VII. Making IL-BKW; Mimetics

DNA which encodes the IL-BKW or fragments thereof can be obtained by chemical synthesis, screening cDNA libraries, or screening genomic libraries prepared from a wide variety of cell lines or tissue samples. See, e.g., Okayama and Berg (1982) Mol. Cell. Biol. 2:161-170; Gubler and Hoffman (1983) Gene 25:263-269; and Glover (ed.) (1984) DNA Cloning: A Practical Approach, IRL Press, Oxford. Alternatively, the sequences provided herein provide useful PCR primers or allow synthetic or other preparation of suitable genes encoding an IL-BKW; including naturally occurring embodiments.

This DNA can be expressed in a wide variety of host cells for the synthesis of a full-length IL-BKW or fragments which can in turn, e.g., be used to generate polyclonal or monoclonal antibodies; for binding studies; for construction and expression of modified molecules; and for structure/function studies.

Vectors, as used herein, comprise plasmids, viruses, bacteriophage, integratable DNA fragments, and other vehicles which enable the integration of DNA fragments into the genome of the host. See, e.g., Pouwels, et al. (1985 and Supplements) Cloning Vectors: A Laboratory Manual, Elsevier, N.Y.; and Rodriguez, et al. (1988)(eds.) Vectors:

A Survey of Molecular Cloning Vectors and Their Uses, Buttersworth, Boston, MA.

For purposes of this invention, DNA sequences are operably linked when they are functionally related to each other. For example, DNA for a presequence or secretory leader is operably linked to a polypeptide if it is expressed as a preprotein or participates in directing the polypeptide to the cell membrane or in secretion of the polypeptide. A promoter is operably linked to a coding sequence if it controls the transcription of the polypeptide; a ribosome binding site is operably linked to a coding sequence if it is positioned to permit translation. Usually, operably linked means contiguous and in reading frame, however, certain genetic elements such as repressor genes are not contiguously linked but still bind to operator sequences that in turn control expression. See, e.g., Rodriguez, et al., Chapter 10, pp. 205-236; Balbas and Bolivar (1990) Methods in Enzymology 185:14-37; and Ausubel, et al. (1993) Current Protocols in Molecular Biology, Greene and Wiley, NY.

Representative examples of suitable expression vectors include pCDNA1; pCD, see Okayama, et al. (1985) Mol. Cell Biol. 5:1136-1142; pMC1neo Poly-A, see Thomas, et al. (1987) Cell 51:503-512; and a baculovirus vector such as pAC 373 or pAC 610. See, e.g., Miller (1988) Ann. Rev. Microbiol. 42:177-199.

It will often be desired to express an IL-BKW polypeptide in a system which provides a specific or defined glycosylation pattern. See, e.g., Luckow and Summers (1988) Bio/Technology 6:47-55; and Kaufman (1990) Meth. Enzymol. 185:487-511.

The IL-BKW, or a fragment thereof, may be engineered to be phosphatidyl inositol (PI) linked to a cell membrane, but can be removed from membranes by treatment with a phosphatidyl inositol cleaving enzyme, e.g., phosphatidyl inositol phospholipase-C. This releases the antigen in a

biologically active form, and allows purification by standard procedures of protein chemistry. See, e.g., Low (1989) Biochim. Biophys. Acta 988:427-454; Tse, et al. (1985) Science 230:1003-1008; and Brunner, et al. (1991) J. Cell Biol. 114:1275-1283.

Now that the IL-BKW has been characterized, fragments or derivatives thereof can be prepared by conventional processes for synthesizing peptides. These include processes such as are described in Stewart and Young (1984) Solid Phase Peptide Synthesis, Pierce Chemical Co., Rockford, IL; Bodanszky and Bodanszky (1984) The Practice of Peptide Synthesis, Springer-Verlag, New York; Bodanszky (1984) The Principles of Peptide Synthesis, Springer-Verlag, New York; and Villafranca (ed.) (1991) Techniques in Protein Chemistry II, Academic Press, San Diego, Ca.

VIII. Uses

The present invention provides reagents which will find use in diagnostic applications as described elsewhere herein, e.g., in IL-BKW mediated conditions, or below in the description of kits for diagnosis.

This invention also provides reagents with significant therapeutic potential. The IL-BKW (naturally occurring or recombinant), fragments thereof, and antibodies thereto, along with compounds identified as having binding affinity to IL-BKW, should be useful in the treatment of conditions associated with abnormal physiology or development, including inflammatory conditions. In particular, modulation of physiology of lymphoid cells will be achieved by appropriate therapeutic treatment using the compositions provided herein. For example, a disease or disorder associated with abnormal expression or abnormal signaling by an IL-BKW should be a likely target for an agonist or antagonist. The new cytokine should play a role in regulation or development of hematopoietic cells, e.g., lymphoid cells, which affect immunological responses, e.g., inflammation and/or autoimmune disorders.

In particular, the cytokine should mediate, in various contexts, cytokine synthesis by the cells, proliferation, etc. Antagonists of IL-BKW, such as mutein variants of a naturally occurring form of IL-BKW or blocking antibodies, may provide a selective and powerful way to block immune responses, e.g., in situations as inflammatory or autoimmune responses, including rheumatoid arthritis, systemic lupus erythematosis (SLE), Hashimoto's autoimmune thyroiditis, as well as acute and chronic inflammatory responses, e.g., inflammatory bowel disease. See also Samter, et al. (eds.) Immunological Diseases vols. 1 and 2, Little, Brown and Co. Modulated cytokine release by the naturally occurring secreted form of IL-BKW, which can be produced in large quantities by recombinant methods, or by blocking antibodies, should be regulatable by reagents made available herein, e.g., in a transplantation rejection situation.

In addition, certain combination compositions would be useful, e.g., with other modulators of inflammation. Such other molecules may include steroids, other versions of IL-10, including cellular species variants, or viral IL-10s, e.g., EBV or EHV, and all of their respective antagonists.

Various abnormal conditions are known in each of the cell types shown to produce IL-BKW mRNA by Northern blot analysis. See Berkow (ed.) The Merck Manual of Diagnosis and Therapy, Merck & Co., Rahway, N.J.; Thorn, et al. Harrison's Principles of Internal Medicine, McGraw-Hill, N.Y.; and Weatherall, et al. (eds.) Oxford Textbook of Medicine, Oxford University Press, Oxford. Many other medical conditions and diseases involve activation by macrophages or monocytes, and many of these will be responsive to treatment by an agonist or antagonist provided herein. See, e.g., Stites and Terr (eds. 1991) Basic and Clinical Immunology Appleton and Lange, Norwalk, Connecticut; and Samter, et al. (eds.) Immunological Diseases Little, Brown and Co. These problems should be

susceptible to prevention or treatment using compositions provided herein.

IL-BKW, antagonists, antibodies, etc., can be purified and then administered to a patient, veterinary or human. These reagents can be combined for therapeutic use with additional active or inert ingredients, e.g., in conventional pharmaceutically acceptable carriers or diluents, e.g., immunogenic adjuvants, along with physiologically innocuous stabilizers, excipients, or preservatives. These combinations can be sterile filtered and placed into dosage forms as by lyophilization in dosage vials or storage in stabilized aqueous preparations. This invention also contemplates use of antibodies or binding fragments thereof, including forms which are not complement binding.

Drug screening using IL-BKW or fragments thereof can be performed to identify compounds having binding affinity to or other relevant biological effects on IL-BKW functions, including isolation of associated components. Subsequent biological assays can then be utilized to determine if the compound has intrinsic stimulating activity and is therefore a blocker or antagonist in that it blocks the activity of the cytokine. Likewise, a compound having intrinsic stimulating activity can activate the signal pathway and is thus an agonist in that it simulates the activity of IL-BKW. This invention further contemplates the therapeutic use of blocking antibodies to IL-BKW as antagonists and of stimulatory antibodies as agonists. This approach should be particularly useful with other IL-BKW species variants.

In addition, IL-BKW may play a role in leukemogenesis or in viral infections by, e.g., HTLV or herpesviruses. It is induced by infection with herpesvirus saimiri. The herpesvirus also encodes a homolog of the cytokine IL-17 (CTLA-8). Thus, the cytokine, or antagonists, may be useful in anti-tumor therapy. The viral correlation may suggest that the cytokine may be important in viral infection or

proliferation processes, or oncology processes, e.g., oncogenic transformation and proliferative conditions, as cancers or leukemias. See, e.g., Thorn, et al. Harrison's Principles of Internal Medicine, McGraw-Hill, N.Y.

In addition, the cytokine appears to be expressed in kidney cell, and may play a significant role in that organ's function, e.g., ion exchange or blood pressure regulation. The cytokine may also have important water balance functions. The cytokine appears to have some expression in kidney.

The quantities of reagents necessary for effective therapy will depend upon many different factors, including means of administration, target site, physiological state of the patient, and other medicants administered. Thus, treatment dosages should be titrated to optimize safety and efficacy. Typically, dosages used in vitro may provide useful guidance in the amounts useful for *in situ* administration of these reagents. Animal testing of effective doses for treatment of particular disorders will provide further predictive indication of human dosage. Various considerations are described, e.g., in Gilman, et al. (eds.) (1990) Goodman and Gilman's: The Pharmacological Bases of Therapeutics, 8th Ed., Pergamon Press; and Remington's Pharmaceutical Sciences, 17th ed. (1990), Mack Publishing Co., Easton, Penn. Methods for administration are discussed therein and below, e.g., for oral, intravenous, intraperitoneal, or intramuscular administration, transdermal diffusion, and others. Pharmaceutically acceptable carriers will include water, saline, buffers, and other compounds described, e.g., in the Merck Index, Merck & Co., Rahway, New Jersey. Dosage ranges would ordinarily be expected to be in amounts lower than 1 mM concentrations, typically less than about 10 μM concentrations, usually less than about 100 nM, preferably less than about 10 pM (picomolar), and most preferably less than about 1 fM (femtomolar), with an appropriate carrier.

Slow release formulations, or a slow release apparatus will often be utilized for continuous or long term administration. See, e.g., Langer (1990) Science 249:1527-1533.

IL-BKW, fragments thereof, and antibodies to it or its fragments, antagonists, and agonists, may be administered directly to the host to be treated or, depending on the size of the compounds, it may be desirable to conjugate them to carrier proteins such as ovalbumin or serum albumin prior to their administration. Therapeutic formulations may be administered in many conventional dosage formulations. While it is possible for the active ingredient to be administered alone, it is preferable to present it as a pharmaceutical formulation. Formulations typically comprise at least one active ingredient, as defined above, together with one or more acceptable carriers thereof. Each carrier should be both pharmaceutically and physiologically acceptable in the sense of being compatible with the other ingredients and not injurious to the patient. Formulations include those suitable for oral, rectal, nasal, topical, or parenteral (including subcutaneous, intramuscular, intravenous and intradermal) administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. See, e.g., Gilman, et al. (eds.) (1990) Goodman and Gilman's: The Pharmacological Bases of Therapeutics, 8th Ed., Pergamon Press; and Remington's Pharmaceutical Sciences, 17th ed. (1990), Mack Publishing Co., Easton, Penn.; Avis, et al. (eds.) (1993) Pharmaceutical Dosage Forms: Parenteral Medications, Dekker, New York; Lieberman, et al. (eds.) (1990) Pharmaceutical Dosage Forms: Tablets, Dekker, New York; and Lieberman, et al. (eds.) (1990) Pharmaceutical Dosage Forms: Disperse Systems, Dekker, New York. The therapy of this invention may be combined with or used in association with other

agents, e.g., other types of IL-10s, or their respective antagonists.

Both the naturally occurring and the recombinant form of the IL-BKWs of this invention are particularly useful in kits and assay methods which are capable of screening compounds for binding activity to the proteins. Several methods of automating assays have been developed in recent years so as to permit screening of tens of thousands of compounds in a short period. See, e.g., Fodor, et al. (1991) Science 251:767-773, which describes means for testing of binding affinity by a plurality of defined polymers synthesized on a solid substrate. The development of suitable assays can be greatly facilitated by the availability of large amounts of purified, soluble IL-BKW as provided by this invention.

Other methods can be used to determine the critical residues in the IL-BKW-IL-BKW receptor interactions. Mutational analysis can be performed, e.g., see Somoza, et al. (1993) J. Exptl. Med. 178:549-558, to determine specific residues critical in the interaction and/or signaling. However, residues in the A and D helices are likely to be most important in receptor interaction.

For example, antagonists can normally be found once the antigen has been structurally defined, e.g., by tertiary structure data. Testing of potential interacting analogs is now possible upon the development of highly automated assay methods using a purified IL-BKW. In particular, new agonists and antagonists will be discovered by using screening techniques described herein. Of particular importance are compounds found to have a combined binding affinity for a spectrum of IL-BKW molecules, e.g., compounds which can serve as antagonists for species variants of IL-BKW.

One method of drug screening utilizes eukaryotic or prokaryotic host cells which are stably transformed with recombinant DNA molecules expressing an IL-BKW. Cells may

be isolated which express an IL-BKW in isolation from other molecules. Such cells, either in viable or fixed form, can be used for standard binding partner binding assays. See also, Parce, et al. (1989) Science 246:243-247; and Owicki, et al. (1990) Proc. Nat'l Acad. Sci. USA 87:4007-4011, which describe sensitive methods to detect cellular responses.

Another technique for drug screening involves an approach which provides high throughput screening for compounds having suitable binding affinity to an IL-BKW and is described in detail in Geysen, European Patent Application 84/03564, published on September 13, 1984. First, large numbers of different small peptide test compounds are synthesized on a solid substrate, e.g., plastic pins or some other appropriate surface, see Fodor, et al. (1991). Then all the pins are reacted with solubilized, unpurified or solubilized, purified IL-BKW, and washed. The next step involves detecting bound IL-BKW.

Rational drug design may also be based upon structural studies of the molecular shapes of the IL-BKW and other effectors or analogs. Effectors may be other proteins which mediate other functions in response to binding, or other proteins which normally interact with IL-BKW, e.g., a receptor. One means for determining which sites interact with specific other proteins is a physical structure determination, e.g., x-ray crystallography or 2 dimensional NMR techniques. These will provide guidance as to which amino acid residues form molecular contact regions, as modeled, e.g., against cellular IL-10. For a detailed description of protein structural determination, see, e.g., Blundell and Johnson (1976) Protein Crystallography, Academic Press, New York.

IX. Kits

This invention also contemplates use of IL-BKW proteins, fragments thereof, peptides, and their fusion products in a variety of diagnostic kits and methods for detecting the presence of another IL-BKW or binding partner.

Typically the kit will have a compartment containing either a defined IL-BKW peptide or gene segment or a reagent which recognizes one or the other, e.g., IL-BKW fragments or antibodies.

A kit for determining the binding affinity of a test compound to an IL-BKW would typically comprise a test compound; a labeled compound, for example a binding partner or antibody having known binding affinity for IL-BKW; a source of IL-BKW (naturally occurring or recombinant); and a means for separating bound from free labeled compound, such as a solid phase for immobilizing the molecule. Once compounds are screened, those having suitable binding affinity to the antigen can be evaluated in suitable biological assays, as are well known in the art, to determine whether they act as agonists or antagonists to the IL-BKW signaling pathway. The availability of recombinant IL-BKW polypeptides also provide well defined standards for calibrating such assays.

A preferred kit for determining the concentration of, e.g., an IL-BKW in a sample would typically comprise a labeled compound, e.g., binding partner or antibody, having known binding affinity for the antigen, a source of cytokine (naturally occurring or recombinant) and a means for separating the bound from free labeled compound, e.g., a solid phase for immobilizing the IL-BKW. Compartments containing reagents, and instructions, will normally be provided.

Antibodies, including antigen binding fragments, specific for the IL-BKW or fragments are useful in diagnostic applications to detect the presence of elevated levels of IL-BKW and/or its fragments. Such diagnostic assays can employ lysates, live cells, fixed cells, immunofluorescence, cell cultures, body fluids, and further can involve the detection of antigens related to the antigen in serum, or the like. Diagnostic assays may be homogeneous (without a separation step between free reagent and antigen-

binding partner complex) or heterogeneous (with a separation step). Various commercial assays exist, such as radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), enzyme immunoassay (EIA), enzyme-multiplied immunoassay technique (EMIT), substrate-labeled fluorescent immunoassay (SLFIA), and the like. See, e.g., Van Vunakis, et al. (1980) Meth Enzymol. 70:1-525; Harlow and Lane (1980) Antibodies: A Laboratory Manual, CSH Press, NY; and Coligan, et al. (eds.) (1993) Current Protocols in Immunology, Greene and Wiley, NY.

Anti-idiotypic antibodies may have similar use to diagnose presence of antibodies against an IL-BKW, as such may be diagnostic of various abnormal states. For example, overproduction of IL-BKW may result in production of various immunological reactions which may be diagnostic of abnormal physiological states, particularly in proliferative cell conditions such as cancer or abnormal activation or differentiation.

Frequently, the reagents for diagnostic assays are supplied in kits, so as to optimize the sensitivity of the assay. For the subject invention, depending upon the nature of the assay, the protocol, and the label, either labeled or unlabeled antibody or binding partner, or labeled IL-BKW is provided. This is usually in conjunction with other additives, such as buffers, stabilizers, materials necessary for signal production such as substrates for enzymes, and the like. Preferably, the kit will also contain instructions for proper use and disposal of the contents after use. Typically the kit has compartments for each useful reagent. Desirably, the reagents are provided as a dry lyophilized powder, where the reagents may be reconstituted in an aqueous medium providing appropriate concentrations of reagents for performing the assay.

Many of the aforementioned constituents of the drug screening and the diagnostic assays may be used without modification or may be modified in a variety of ways. For

example, labeling may be achieved by covalently or non-covalently joining a moiety which directly or indirectly provides a detectable signal. In any of these assays, the binding partner, test compound, IL-BKW, or antibodies thereto can be labeled either directly or indirectly. Possibilities for direct labeling include label groups: radiolabels such as ^{125}I, enzymes (U.S. Pat. No. 3,645,090) such as peroxidase and alkaline phosphatase, and fluorescent labels (U.S. Pat. No. 3,940,475) capable of monitoring the change in fluorescence intensity, wavelength shift, or fluorescence polarization. Possibilities for indirect labeling include biotinylation of one constituent followed by binding to avidin coupled to one of the above label groups.

There are also numerous methods of separating the bound from the free IL-BKW, or alternatively the bound from the free test compound. The IL-BKW can be immobilized on various matrixes followed by washing. Suitable matrixes include plastic such as an ELISA plate, filters, and beads. See, e.g., Coligan, et al. (eds.) (1993) Current Protocols in Immunology, Vol. 1, Chapter 2, Greene and Wiley, NY. Other suitable separation techniques include, without limitation, the fluorescein antibody magnetizable particle method described in Rattle, et al. (1984) Clin. Chem. 30:1457-1461, and the double antibody magnetic particle separation as described in U.S. Pat. No. 4,659,678.

Methods for linking proteins or their fragments to the various labels have been extensively reported in the literature and do not require detailed discussion here. Many of the techniques involve the use of activated carboxyl groups either through the use of carbodiimide or active esters to form peptide bonds, the formation of thioethers by reaction of a mercapto group with an activated halogen such as chloroacetyl, or an activated olefin such as maleimide, for linkage, or the like. Fusion proteins will also find use in these applications.

Another diagnostic aspect of this invention involves use of oligonucleotide or polynucleotide sequences taken from the sequence of an IL-BKW. These sequences can be used as probes for detecting levels of the IL-BKW message in samples from patients suspected of having an abnormal condition, e.g., inflammatory or autoimmune. Since the cytokine may be a marker or mediator for activation, it may be useful to determine the numbers of activated cells to determine, e.g., when additional therapy may be called for, e.g., in a preventative fashion before the effects become and progress to significance. The preparation of both RNA and DNA nucleotide sequences, the labeling of the sequences, and the preferred size of the sequences has received ample description and discussion in the literature. See, e.g., Langer-Safer, et al. (1982) Proc. Nat'l. Acad. Sci. 79:4381-4385; Caskey (1987) Science 236:962-967; and Wilchek et al. (1988) Anal. Biochem. 171:1-32.

Diagnostic kits which also test for the qualitative or quantitative expression of other molecules are also contemplated. Diagnosis or prognosis may depend on the combination of multiple indications used as markers. Thus, kits may test for combinations of markers. See, e.g., Viallet, et al. (1989) Progress in Growth Factor Res. 1:89-97. Other kits may be used to evaluate other cell subsets.

X. Isolating the IL-BKW Receptor

Having isolated a ligand of a specific ligand-receptor interaction, methods exist for isolating the receptor. See, Gearing, et al. (1989) EMBO J. 8:3667-3676. For example, means to label the IL-BKW cytokine without interfering with the binding to its receptor can be determined. For example, an affinity label can be fused to either the amino- or carboxyl-terminus of the ligand, though based on IL-10, the amino-terminus is more likely to succeed. Such label may be a FLAG epitope tag, or, e.g., an Ig or Fc domain. An expression library can be screened for specific binding of the cytokine, e.g., by cell sorting, or other screening to

detect subpopulations which express such a binding component. See, e.g., Ho, et al. (1993) Proc. Nat'l Acad. Sci. USA 90:11267-11271; and Liu, et al. (1994) J. Immunol. 152:1821-29. Alternatively, a panning method may be used. See, e.g., Seed and Aruffo (1987) Proc. Nat'l Acad. Sci. USA 84:3365-3369.

Protein cross-linking techniques with label can be applied to isolate binding partners of the IL-BKW cytokine. This would allow identification of proteins which specifically interact with the cytokine, e.g., in a ligand-receptor like manner.

Early experiments will be performed to determine whether the known IL-10R is involved in response(s) to IL-BKW. It is also quite possible that the functional IL-10 receptor complex may share many or all components with an IL-BKW receptor complex, either a specific receptor subunit or an accessory receptor subunit.

Many modifications and variations of this invention can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. The specific embodiments described herein are offered by way of example only, and the invention is to be limited only by the terms of the appended claims, along with the full scope of equivalents to which such claims are entitled.

EXAMPLES

General Methods

Some of the standard methods are described or referenced, e.g., in Maniatis, et al. (1982) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor Press; Sambrook, et al. (1989) Molecular Cloning: A Laboratory Manual (2d ed.), vols. 1-3, CSH Press, NY; Ausubel, et al., Biology, Greene Publishing Associates, Brooklyn, NY; or Ausubel, et al. (1987 and Supplements) Current Protocols in Molecular Biology, Greene and Wiley, New York; Innis, et al. (eds.)(1990) PCR Protocols: A Guide

to Methods and Applications, Academic Press, N.Y. Methods for protein purification include such methods as ammonium sulfate precipitation, column chromatography, electrophoresis, centrifugation, crystallization, and others. See, e.g., Ausubel, et al. (1987 and periodic supplements); Deutscher (1990) "Guide to Protein Purification" in Methods in Enzymology vol. 182, and other volumes in this series; and manufacturer's literature on use of protein purification products, e.g., Pharmacia, Piscataway, N.J., or Bio-Rad, Richmond, CA. Combination with recombinant techniques allow fusion to appropriate segments, e.g., to a FLAG sequence or an equivalent which can be fused via a protease-removable sequence. See, e.g., Hochuli (1989) Chemische Industrie 12:69-70; Hochuli (1990) "Purification of Recombinant Proteins with Metal Chelate Absorbent" in Setlow (ed.) Genetic Engineering, Principle and Methods 12:87-98, Plenum Press, N.Y.; and Crowe, et al. (1992) QIAexpress: The High Level Expression & Protein Purification System QUIAGEN, Inc., Chatsworth, CA. Cell culture techniques are described in Doyle, et al. (eds.) (1994) Cell and Tissue Culture: Laboratory Procedures, John Wiley and Sons, NY.

Standard immunological techniques are described, e.g., in Hertzenberg, et al. (eds. 1996) Weir's Handbook of Experimental Immunology vols. 1-4, Blackwell Science; Coligan (1991) Current Protocols in Immunology Wiley/Greene, NY; and Methods in Enzymology volumes. 70, 73, 74, 84, 92, 93, 108, 116, 121, 132, 150, 162, and 163.

FACS analyses are described in Melamed, et al. (1990) Flow Cytometry and Sorting Wiley-Liss, Inc., New York, NY; Shapiro (1988) Practical Flow Cytometry Liss, New York, NY; and Robinson, et al. (1993) Handbook of Flow Cytometry Methods Wiley-Liss, New York, NY. Fluorescent labeling of appropriate reagents was performed by standard methods.

EXAMPLE 1: Cloning of human and mouse IL-BKW

PBMC are prepared from a healthy human blood donor by conventional Ficoll gradients, as described, e.g., in Coligan, et al. Current Protocols in Immunology Greene/Wiley. Cells, preferably monocytes, from this preparation are stimulated, e.g., with PHA. RNA from these activated monocytes is used to isolate a cDNA based upon the sequence information about the mda7.

PCR products are cloned using a TA cloning kit (Invitrogen). The resulting cDNA plasmids are sequenced from both termini on an automated sequencer (Applied Biosystems).

The clone for mIL-BKW was isolated from an NK1.1+, CD4+ activated mouse thymocyte cDNA library (A. Zlotnik, DNAX Research Institute, Palo Alto, CA). Sequencing of this clone reveal high amino acid sequence identity to hIL-BKW as well as mIL-10 and hIL-10. In particular, a high degree of identity between the four cytokines was found in the D-helix domain, implicated in receptor binding.

EXAMPLE 2: Cellular Expression of Mammalian IL-BKW

Because of the sequence similarity to human IL-10, distribution will be investigated for similar type cell types. A probe specific for cDNA encoding primate IL-BKW is labeled, e.g., by random priming.

IL-BKW/ak155 is strongly transcribed in various T-cell tissues and clones.

Southern Analysis: DNA (5 µg) from the primary amplified cDNA library was digested with appropriate restriction enzymes to release the inserts, run on a 1% agarose gel and transferred to a nylon membrane (Schleicher and Schuell, Keene, NH).

Multiple tissue Northern blots were used to determine the size and tissue distribution of hIL-BKW mRNA. A number of mRNA transcipts, varying in size from 1.0 kb to 4.4 kb, were detected in most of the tissues. Based on the stringency of the hybridization and wash conditions of the

blots, these transcripts most likely represent alternatively spliced mRNA's and not different family members. A similar Northern blot using cancer cell lines (Clontech) revealed a strongly hybridizing 2.2 kb mRNA transcript in colorectal carcinoma SW480, but that was not present in any of the other cancer lines.

The expression profile of hIL-BKW in monocytes/macrophages, dendritic, T, B, and NK cells was analysed by cDNA library Southern blot hybridization. The hIL-BKW probe was hybridized to 5 μg of each cDNA library following digestion with NotI and SalI to release the cDNA inserts from the pSport vector (Gibco-BRL). Among T, B, and NK cells, expession of hIL-BKW was restricted to an activated Th0 clone (Mot72) and an activated Th1 clone (HY06). By far the highest level of hIL-BKW expression was seen in elutriated monocytes activated with LPS, IFN-γ and anti-IL-10 antibodies. In contrast, when we probed a library made from these same monocytes treated with IL-10 instead of anti-IL10, a sharp decrease in the level of IL-BKW expression was observed, suggesting that expression of IL-BKW is strongly regulated by IL-10.

Samples for mRNA isolation for analysis of mIL-BKW include: resting mouse fibroblastic L cell line (C200); Braf:ER (Braf fusion to estrogen receptor) transfected cells, control (C201); T cells, TH1 polarized (Mel14 bright, CD4+ cells from spleen, polarized for 7 days with IFN-γ and anti IL-4; T200); T cells, TH2 polarized (Mel14 bright, CD4+ cells from spleen, polarized for 7 days with IL-4 and anti-IFN-γ; T201); T cells, highly TH1 polarized (see Openshaw, et al. (1995) J. Exp. Med. 182:1357-1367; activated with anti-CD3 for 2, 6, 16 h pooled; T202); T cells, highly TH2 polarized (see Openshaw, et al. (1995) J. Exp. Med. 182:1357-1367; activated with anti-CD3 for 2, 6, 16 h pooled; T203); CD44- CD25+ pre T cells, sorted from thymus (T204); TH1 T cell clone D1.1, resting for 3 weeks after last stimulation with antigen (T205); TH1 T cell clone D1.1,

10 μg/ml ConA stimulated 15 h (T206); TH2 T cell clone CDC35, resting for 3 weeks after last stimulation with antigen (T207); TH2 T cell clone CDC35, 10 μg/ml ConA stimulated 15 h (T208); Mel14+ naive T cells from spleen, resting (T209); Mel14+ T cells, polarized to Th1 with IFN-γ/IL-12/anti-IL-4 for 6, 12, 24 h pooled (T210); Mel14+ T cells, polarized to Th2 with IL-4/anti-IFN-γ for 6, 13, 24 h pooled (T211); unstimulated mature B cell leukemia cell line A20 (B200); unstimulated B cell line CH12 (B201); unstimulated large B cells from spleen (B202); B cells from total spleen, LPS activated (B203); metrizamide enriched dendritic cells from spleen, resting (D200); dendritic cells from bone marrow, resting (D201); monocyte cell line RAW 264.7 activated with LPS 4 h (M200); bone-marrow macrophages derived with GM and M-CSF (M201); macrophage cell line J774, resting (M202); macrophage cell line J774 + LPS + anti-IL-10 at 0.5, 1, 3, 6, 12 h pooled (M203); macrophage cell line J774 + LPS + IL-10 at 0.5, 1, 3, 5, 12 h pooled (M204); aerosol challenged mouse lung tissue, Th2 primed, aerosol OVA challenge 7, 14, 23 h pooled (see Garlisi, et al. (1995) Clinical Immunology and Immunopathology 75:75-83; X206); Nippostrongulus-infected lung tissue (see Coffman, et al. (1989) Science 245:308-310; X200); total adult lung, normal (O200); total lung, rag-1 (see Schwarz, et al. (1993) Immunodeficiency 4:249-252; O205); IL-10 K.O. spleen (see Kuhn, et al. (1991) Cell 75:263-274; X201); total adult spleen, normal (O201); total spleen, rag-1 (O207); IL-10 K.O. Peyer's patches (O202); total Peyer's patches, normal (O210); IL-10 K.O. mesenteric lymph nodes (X203); total mesenteric lymph nodes, normal (O211); IL-10 K.O. colon (X203); total colon, normal (O212); NOD mouse pancreas (see Makino, et al. (1980) Jikken Dobutsu 29:1-13; X205); total thymus, rag-1 (O208); total kidney, rag-1 (O209); total heart, rag-1 (O202); total brain, rag-1 (O203); total testes, rag-1 (O204); total liver, rag-1 (O206); rat normal joint tissue (O300); and rat arthritic joint tissue (X300).

Expression by cDNA Southern analysis was very high in T cells, TH2 polarized (Mel14 bright, CD4+ cells from spleen, polarized for 7 days with IL-4 and anti-IFN-γ; T201); high in T cells, highly TH2 polarized (see Openshaw, et al. (1995) J. Exp. Med. 182:1357-1367; activated with anti-CD3 for 2, 6, 16 h pooled; T203); and significant in: T cells, TH1 polarized (Mel14 bright, CD4+ cells from spleen, polarized for 7 days with IFN-γ and anti IL-4; T200); and Mel14+ T cells, polarized to Th2 with IL-4/anti-IFN-γ for 6, 13, 24 h pooled (T211).

EXAMPLE 3: Chromosome mapping of mouse and human IL-BKW

The mIL-BKW gene was mapped to mouse chromosome 1 using procedures well known in the art. See, e.g., Copeland, et al., (1993) Science 262:57-66. mIL-10 maps directly adjacent to mIL-BKW on chromosome 1.

To map the human counterpart, an isolated human IL-BKW cDNA encoding the IL-BKW is used. Chromosome mapping is a standard technique as described above. See, e.g., BIOS Laboratories (New Haven, CT) and methods for using a mouse somatic cell hybrid panel with PCR. The human gene has been mapped to human Chromosome 1, and is likely, from mouse syntenic information, to the 1q32 region.

EXAMPLE 4: Purification of IL-BKW Protein

Multiple transfected cell lines are screened for one which expresses the cytokine at a high level compared with other cells. Various cell lines are screened and selected for their favorable properties in handling. Natural IL-BKW can be isolated from natural sources, or by expression from a transformed cell using an appropriate expression vector. Purification of the expressed protein is achieved by standard procedures, or may be combined with engineered means for effective purification at high efficiency from cell lysates or supernatants. FLAG or His_6 segments can be used for such purification features. Alternatively,

affinity chromatography may be used with specific antibodies, see below.

Protein is produced in coli, insect cell, or mammalian expression systems. Production of mouse fusion protein as an IgG fusion, with an IGase cleavage site, in COP5 cells resulted in secreted product which was N-terminal blocked. Microsequencing analysis indicated that the actual N-terminus was QEF.... Production of similar material with human sequence provides an N-terminus of AQG....

Recombinant human IL-BKW produced from a standard construct in mammalian cells seems to be found only in small amounts as a soluble form in the medium. Evidence exists that the protein binds tightly to cell surface proteoglycans, and is difficult to solubilize. Heparin treatment causes some release of that protein. Surface proteoglycan deficient cells may be used to produce the protein.

EXAMPLE 5: Isolation of Homologous IL-BKW Genes

The IL-BKW cDNA can be used as a hybridization probe to screen a library from a desired source, e.g., a primate cell cDNA library. Many different species can be screened both for stringency necessary for easy hybridization, and for presence using a probe. Appropriate hybridization conditions will be used to select for clones exhibiting specificity of cross hybridization.

Screening by hybridization using degenerate probes based upon the peptide sequences will also allow isolation of appropriate clones. Alternatively, use of appropriate primers for PCR screening will yield enrichment of appropriate nucleic acid clones.

Similar methods are applicable to isolate either species, polymorphic, or allelic variants. Species variants are isolated using cross-species hybridization techniques based upon isolation of a full length isolate or fragment from one species as a probe.

Alternatively, antibodies raised against human IL-BKW will be used to screen for cells which express cross-reactive proteins from an appropriate, e.g., cDNA library. The purified protein or defined peptides are useful for generating antibodies by standard methods, as described above. Synthetic peptides or purified protein are presented to an immune system to generate monoclonal or polyclonal antibodies. See, e.g., Coligan (1991) Current Protocols in Immunology Wiley/Greene; and Harlow and Lane (1989) Antibodies: A Laboratory Manual Cold Spring Harbor Press. The resulting antibodies are used for screening, purification, or diagnosis, as described.

EXAMPLE 6: Preparation of antibodies specific for IL-BKW

Synthetic peptides or purified protein are presented to an immune system to generate monoclonal or polyclonal antibodies. See, e.g., Coligan (1991) Current Protocols in Immunology Wiley/Greene; and Harlow and Lane (1989) Antibodies: A Laboratory Manual Cold Spring Harbor Press. Polyclonal serum, or hybridomas may be prepared. In appropriate situations, the binding reagent is either labeled as described above, e.g., fluorescence or otherwise, or immobilized to a substrate for panning methods.

A number of monoclonal antibodies were obtained. Of those which were purified; half were IgG and the other half were IgM. Most of these were good Western blot reagents. Several of the others stain recombinant hIL-BKW expressed on the cell surface, and others were adequate for immunohistochemistry. "Positive" cells seen via the latter in tonsil sections appear to be macrophage-like.

EXAMPLE 7: IL-10 regulated expression of IL-BKW

To investigate the regulatory effect of IL-10 directly by Northern blot analysis, mRNA was prepared from

elutriated, LPS and IFN-γ activated monocytes, treated with either IL-10 or an anti-IL-10 antibody. Just as assessed by Southerns blots of the cDNA libraries, strong induction of IL-BKW mRNA was observed in activated monocytes treated with anti-IL-10, whereas treatment of these cells under identical conditions in the presence of IL-10 almost completely shut down synthesis of IL-BKW message.

EXAMPLE 8: Evaluation of Breadth of Biological Functions

Biological activities of IL-BKW were tested based on the sequence and structural homology between IL-BKW and IL-10. Initially, assays that had shown biological activities of IL-10 were examined. These include assays on human peripheral blood mononuclear cells, human monocytes, and human T cell clones.

A. Effects on the expression of cell surface molecules on human monocytes.

Monocytes were purified by negative selection from peripheral blood mononuclear cells of normal healthy donors. Briefly, 3×10^8 ficoll banded mononuclear cells were incubated on ice with a cocktail of monoclonal antibodies (Becton-Dickenson; Mountain View, CA) consisting of 200 µl of αCD2 (Leu-5A), 200 µl of αCD3 (Leu-4), 100 µl of αCD8 (Leu 2a), 100 µl of αCD19 (Leu-12), 100 µl of αCD20 (Leu-16), 100 µl of αCD56 (Leu-19), 100 µl of αCD67 (IOM 67) (Immunotech,Westbrook, ME), and anti-glycophorin antibody (10F7MN, ATCC, Rockville, MD). Antibody bound cells were washed and then incubated with sheep anti-mouse IgG coupled magnetic beads (Dynal, Oslo, Norway) at a bead to cell ratio of 20:1. Antibody bound cells were separated from monocytes by application of a magnetic field. Subsequently, human monocytes were cultured in Yssel's medium (Gemini Bioproducts, Calabasas, CA) containing 1% human AB serum in the absence or presence of IL-BKW (1/100 dilution baculovirus expressed material or 1 µg/ml mammalian cop 5

expressed material) or IL-10 (200 U/ml) in polypropylene 96 well plates (Costar) for 40 hrs. In addition, identical cultures were established in the presence of IFN-γ (100 U/ml).

Analyses of the expression of cell surface molecules was performed by direct immunofluorescence. Briefly, 2 x 10^5 purified human monocytes were incubated in phosphate buffered saline (PBS) containing 1% human serum on ice for 20 minutes. Cells were pelleted at 200 x g. Cells were resuspended in 20 ml PE or FITC labeled mAb. Following an additional 20 minute incubation on ice, cells were washed in PBS containing 1% human serum followed by two washes in PBS alone. Cells were fixed in PBS containing 1% paraformaldehyde and analyzed on FACScan flow cytometer (Becton Dickenson; Mountain View, CA) and results are expressed as mean fluorescence intensity in Table 3. The following mAbs were used: CD11b (anti-mac1), CD11c (a gp150/95), CD14 (Leu-M3), CD54 (Leu 54), CD80 (anti-BB1/B7), HLA-DR (L243) from Becton-Dickinson and CD86 (FUN 1) (Pharmingen), CD64 (32.2) (Medarex), CD40 (mAb89) (Schering-Plough France).

TABLE 3

Effects of IL-BKW on the cell surface phenotype of human monocytes

Exp 1	CD11B	CD14	CD54	CD80	CD86	HLA-DR	CD40
med-	261	976	159	16/33	395	1522	251
IL-BKW	202	1850	195	39/69	217	495	249
IFN-	307	365	262	93/87	277	3688	697
IFN + IL-BKW	222	275	342	97/268	375	4157	1299
Exp 2							
t=0	668	1414	37	1	58	805	50
med-	293	1455	239	41/32	202	3130	152
IL-BKW	446	607	325	91/66	299	2190	123
IFN-	513	557	605	96/88	227	5664	481
IFN+ IL-BKW	485	529	633	98/181	338	7318	702

IL-BKW enhanced the expression of CD54 (ICAM-1), CD80, and CD86, whereas it decreased the expression of HLA-DR (see Table 3). IL-BKW further enhanced the upregulation of CD40, CD54, CD80, and CD86 by IFN-γ. In the presence of IFN-γ, IL-BKW enhanced the expression of HLA-DR. A variable effect of IL-BKW alone was observed on the expression of CD11b, CD11c, and CD14, whereas in combination with IFN-γ it reduced cell surface expression as compared to IFN-γ alone. Since IL-10 downregulates CD54, CD80, CD86, and HLA-DR, both in the presence or absence of IFN-γ, these results indicate that IL-20 has different effects on the expression of cell surface molecules on monocytes as compared to IL-10.

B. Effects of IL-BKW on cytokine production by human monocytes.

Human monocytes were isolated as described and cultured in Yssel's medium (Gemini Bioproducts, Calabasas, CA) containing 1% human AB serum in the absence or presence of IL-BKW (1/100 dilution baculovirus expressed material). In addition, monocytes were stimulated with the indicated amounts of LPS (E. coli 0127:B8 Difco) in the absence or presence of IL-BKW for 24 hrs and the concentration of cytokines (IL-1b, IL-6, TNFα, GM-CSF, and IL-10) in the cell culture supernatant was determined by ELISA. For intracytoplasmic staining for cytokines, monocytes were cultured (1 million/ml) in Yssel's medium in the absence or presence of IL-BKW (1/100 dilution baculovirus expressed material or 1 mg/ml mammalian cop 5 expressed material) and/or 5 mg/ml LPS (E. coli 0127:B8 Difco) and 10 mg/ml Brefeldin A (Epicentre technologies Madison WI) for 12 hrs. Cells were washed in PBS and incubated in 2% formaldehyde/PBS solution for 20 minutes at RT. Subsequently cells were washed, resuspended in permeabilization buffer (0.5% saponin (Sigma) in PBS/BSA (0.5%) /Azide (1 mM)) and incubated for 20 minutes at RT. Cells (2×10^5) were centrifuged and resuspended in 20 ml directly conjugated anti-cytokine mAbs diluted 1:10 in

permeabilization buffer for 20 minutes at RT. The following antibodies were used: IL-1α PE (364-3B3-14); IL-6-PE (MQ2-13A5); TNFα-PE (MAb11); GM-CSF-PE (BVD2-21C11); and IL-12-PE (C11.5.14) (Pharmingen San Diego, CA). Subsequently, cells were washed twice in permeabilization buffer and once in PBS/BSA/Azide and analyzed on FACScan flow cytometer (Becton Dickenson; Mountain View, CA). Results are expressed as % positive cells and mean fluorescence intensity of the positive population in Table 4.

TABLE 4

Effects of IL-BKW on cytokine production by human monocytes (% intracellular staining/mean fluorescence intensity)

	IL-1	IL-6	TNF
Medium	5	5	4
IL-BKW (1/100)	79/300	67/182	44/627
LPS (5 μg/ml)	67/456	78/404	78/1524
LPS + IL-BKW	77/613	75/458	77/1573

	IL-1	IL-6	TNF
medium	9/58	39/65	7/159
IL-BKW (1 μg/ml)	57/93	93/575	70/547
LPS (5 μg/ml)	77/174	94/716	79/498
LPS + IL-BKW	79/172	96/1106	81/725

IL-BKW induced the expression of IL-6, TNFα, IL-10, IL-1b, and GM-CSF by human monocytes (Table 5). LPS also induced the expression of these cytokines in a dose dependent manner. Addition of IL-BKW in combination with LPS resulted in an enhanced production of IL-6, TNFα, IL-10, IL-1b, and GM-CSF as compared to either LPS or IL-BKW alone.

TABLE 5

Effects of IL-BKW on cytokine production by human monocytes

	IL-1b	IL-6	TNF	IL-10	GM-CSF
medium	0	2.229	0	0.007	0
LPS(ng/ml) 1000	0.321	29	4.735	0.867	0.828
100	0	36.78	4.683	1.417	0.371
10	0	37.06	2.156	1.216	0.080
1	0	36.22	0.806	0.918	0
IL-BKW (1/100)	1.460	27.9	1.808	0.391	0.673
+ LPS 1000	4.637	93.02	12.09	2.332	2.048
100	3.921	82.56	9.843	3.994	1.309
10	4.024	142.2	5.708	3.096	1.466
1	2.744	85.66	4.006	2.58	0.852

(results expressed in ng/ml)

Similar results were obtained when cytokine production was assessed by intracytoplasmic staining. IL-BKW induced expression of IL-1, IL-6, and TNFα by human monocytes as indicated by an increase in the percentage of positive cells as well as the fluorescence intensity, which reflects the quantity of cytokine produced per cell. LPS induced the expression of IL-1, IL-6, and TNFα; and addition of IL-BKW to these cultures further increased this production. This enhanced production was observed for both baculovirus and mammalian expressed material. See Table 4.

C. Effects of IL-BKW on cytokine production by human peripheral blood mononuclear cells (PBMC).

Total PBMC were isolated from buffy coats of normal healthy donors by centrifugation through ficoll-hypaque as described (Boyum, et al.) and cultured in Yssel's medium (Gemini Bioproducts, Calabasas, CA) containing 1% human AB serum in the absence or presence of IL-BKW (1/100 dilution baculovirus expressed material). Cells were incubated at 2 x 10^6 cells/ml in medium or activated by PHA (100 ng/ml) or IL-2 (100 U/ml) (R&D Systems). In addition, PBMC were

cultured with IL-10 alone (100 U/ml) or IL-10 in combination with IL-BKW (1/100 dilution baculovirus expressed material). Cytokine secretion was determined in supernatants harvested at 72 hours by cytokine specific ELISA.

IL-BKW induced the expression of IL-6, TNFα, IL-10, and IFN-γ by PBMC (see Table 6). Furthermore, IL-BKW enhanced the production of IL-6, TNFα, IL-10, and IFN-γ when PBMC were activated by PHA or IL-2. IL-10 inhibited the production of IL-6, TNFα, and IFN-γ following activation of PBMC by IL-BKW, PHA, IL-2, or combinations of IL-BKW with either PHA or IL-2.

TABLE 6

Effects of IL-BKW on the cytokine production by PBMC

PBMC	IL-6	TNFα	IL-10	IFN-γ
medium	0.243	0	0.007	0
IL-10 (100 U/ml)	0.196	0	NT	0
IL-BKW (1/100)	24.23	0.029	1.295	0.004
IL-10 + IL-BKW	0.339	0	NT	0
PHA (100 ng/ml)	5.491	0.652	0.637	1.05
IL-10	0.279	0	NT	0.004
IL-BKW	67.06	3.31	4.556	5.742
IL-10 + IL-BKW	1.129	0	NT	0.1
IL-2 (100 U/ml)	41.4	0.216	3.224	0.895
IL-10	0.89	0	NT	0.01
IL-BKW	62.34	0.621	10.04	1.507
IL-10 + IL-BKW	3.456	0	NT	0.081

results expressed in ng/ml; NT: not tested.

D. Effects of IL-BKW on proliferation of human peripheral blood mononuclear cells (PBMC).

Total PBMC were isolated from buffy coats of normal healthy donors by centrifugation through ficoll-hypaque as described (Boyum, et al.). PBMC were cultured in 200 ml Yssel's medium (Gemini Bioproducts, Calabasas, CA) containing 1% human AB serum in 96 well plates (Falcon,

Becton-Dickinson, NJ) in the absence or presence of indicated amounts of IL-BKW (mammalian cop 5 expressed material). Cells were cultured in medium alone or in combination with 100 U/ml IL-2 (R&D Systems) for 120 hours. 3H-Thymidine (0.1 mCi) was added during the last six hours of culture and 3H-Thymidine incorporation was determined by liquid scintillation counting. Results are expressed as mean cpm of triplicate cultures.

IL-BKW induces a low level of proliferation of PBMC in a dose dependent manner. See Table 7. In addition, IL-BKW enhances also in a dose dependent manner the proliferative response of PBMC to IL-2.

TABLE 7

Effect of IL-BKW on proliferation by PBMC

IL-BKW (ng/ml)	MEAN	S.D.	+ IL-2 (100 U/ml) MEAN	S.D.
1000	5894	2655	43426	12858
333	6430	1877	48187	2470
111	3510	1384	36435	5058
37	1016	331	24859	6014
12	235	60	22738	3379
4	190	75	23323	4266
1.3	175	49	22528	2979
0	100	4	17961	3839

results expressed as cpm +/- standard deviation

The native, recombinant, and fusion proteins would be tested for agonist and antagonist activity in many other biological assay systems, e.g., on T-cells, B-cells, NK, macrophages, dendritic cells, hematopoietic progenitors, etc. Because of the IL-10 structural relationship, assays related to IL-10 activity would analyzed

IL-BKW is evaluated for agonist or antagonist activity on transfected cells expressing IL-10 receptor and controls. See, e.g., Ho, et al. (1993) Proc. Nat'l Acad. Sci. USA 90, 11267-11271; Ho, et al. (1995) Mol. Cell. Biol. 15:5043-5053;and Liu, et al. (1994). J. Immunol. 152:1821-1829.

Based, in part, upon the structural homology to IL-10, the IL-BKW is evaluated for effect in macrophage/dendritic cell activation and antigen presentation assays, T cell cytokine production and proliferation in response to antigen or allogeneic stimulus. See, e.g., de Waal Malefyt et al. (1991) J. Exp. Med. 174:1209-1220; de Waal Malefyt et al. (1991) J. Exp. Med. 174:915-924; Fiorentino, et al. (1991) J. Immunol. 147, 3815-3822; Fiorentino, et al. (1991) J. Immunol. 146:3444-3451; and Groux, et al. (1996) J. Exp. Med. 184:19-29.

IL-BKW will also be evaluated for effects on NK cell stimulation. Assays may be based, e.g., on Hsu, et al. (1992) Internat. Immunol. 4:563-569; and Schwarz, et al. (1994) J. Immunother. 16:95-104.

B cell growth and differentiation effects will be analyzed, e.g., by the methodology described, e.g., in Defrance, et al. (1992). J. Exp. Med. 175:671-682; Rousset, et al (1992) Proc. Nat'l Acad. Sci. USA 89:1890-1893; including IgG2 and IgA2 switch factor assays. Note that, unlike COS7 supernatants, NIH3T3 and COP supernatants apparently do not interfere with human B cell assays.

All references cited herein are incorporated herein by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference in its entirety for all purposes.

Many modifications and variations of this invention can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. The specific embodiments described herein are offered by way of example only, and the invention is to be limited only by the terms of the appended claims, along with the full scope of equivalents to which such claims are entitled.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Schering Corporation

(ii) TITLE OF INVENTION: Mammalian Cytokine; Related Reagents

(iii) NUMBER OF SEQUENCES: 7

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Schering-Plough Corporation
(B) STREET: 2000 Galloping Hill Road
(C) CITY: Kenilworth
(D) STATE: NJ
(E) COUNTRY: USA
(F) ZIP: 07033

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: Apple Macintosh
(C) OPERATING SYSTEM: Macintosh 7.1
(D) SOFTWARE: Microsoft Word 7.5.3

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: PCT
(B) FILING DATE: 22-DEC-1997
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 60/034,151
(B) FILING DATE: 23-DEC-1996

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/842,659
(B) FILING DATE: 15-APR-1997

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: FOULKE, CYNTHIA L.
(B) REGISTRATION NUMBER: 32,364
(C) REFERENCE/DOCKET NUMBER: DX0683K

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (908) 298-2987
(B) TELEFAX: (908) 298-5388

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1700 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 275..892

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 419..892

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
CTTGCCTGCA AACCTTTACT TCTGAAATGA CTTCCACGGC TGGGACGGGA ACCTTCCACC      60

CACAGCTATG CCTCTGATTG GTGAATGGTG AAGGTGCCTG TCTAACTTTT CTGTAAAAAG     120

AACCAGCTGC CTCCAGGCAG CCAGCCCTCA AGCATCACTT ACAGGACCAG AGGGACAAGA     180

CATGACTGTG ATGAGGAGCT GCTTTCGCCA ATTTAACACC AAGAAGAATT GAGGCTGCTT     240

GGGAGGAAGG CCAGGAGGAA CACGAGACTG AGAG ATG AAT TTT CAA CAG AGG        292
                                      Met Asn Phe Gln Gln Arg
                                      -48             -45

CTG CAA AGC CTG TGG ACT TTA GCC AGA CCC TTC TGC CCT CCT TTG CTG      340
Leu Gln Ser Leu Trp Thr Leu Ala Arg Pro Phe Cys Pro Pro Leu Leu
        -40                 -35                 -30

GCG ACA GCC TCT CAA ATG CAG ATG GTT GTG CTC CCT TGC CTG GGT TTT      388
Ala Thr Ala Ser Gln Met Gln Met Val Val Leu Pro Cys Leu Gly Phe
    -25                 -20                 -15

ACC CTG CTT CTC TGG AGC CAG GTA TCA GGG GCC CAG GGC CAA GAA TTC      436
Thr Leu Leu Leu Trp Ser Gln Val Ser Gly Ala Gln Gly Gln Glu Phe
-10                 -5                   1               5

CAC TTT GGG CCC TGC CAA GTG AAG GGG GTT GTT CCC CAG AAA CTG TGG      484
His Phe Gly Pro Cys Gln Val Lys Gly Val Val Pro Gln Lys Leu Trp
             10                  15                  20

GAA GCC TTC TGG GCT GTG AAA GAC ACT ATG CAA GCT CAG GAT AAC ATC      532
Glu Ala Phe Trp Ala Val Lys Asp Thr Met Gln Ala Gln Asp Asn Ile
         25                  30                  35

ACG AGT GCC CGG CTG CTG CAG CAG GAG GTT CTG CAG AAC GTC TCG GAT      580
Thr Ser Ala Arg Leu Leu Gln Gln Glu Val Leu Gln Asn Val Ser Asp
     40                  45                  50

GCT GAG AGC TGT TAC CTT GTC CAC ACC CTG CTG GAG TTC TAC TTG AAA      628
Ala Glu Ser Cys Tyr Leu Val His Thr Leu Leu Glu Phe Tyr Leu Lys
 55                  60                  65                  70
```

```
ACT GTT TTC AAA AAC TAC CAC AAT AGA ACA GTT GAA GTC AGG ACT CTG        676
Thr Val Phe Lys Asn Tyr His Asn Arg Thr Val Glu Val Arg Thr Leu
                 75                  80                  85

AAG TCA TTC TCT ACT CTG GCC AAC AAC TTT GTT CTC ATC GTG TCA CAA        724
Lys Ser Phe Ser Thr Leu Ala Asn Asn Phe Val Leu Ile Val Ser Gln
             90                  95                 100

CTG CAA CCC AGT CAA GAA AAT GAG ATG TTT TCC ATC AGA GAC AGT GCA        772
Leu Gln Pro Ser Gln Glu Asn Glu Met Phe Ser Ile Arg Asp Ser Ala
        105                 110                 115

CAC AGG CGG TTT CTG CTA TTC CGG AGA GCA TTC AAA CAG TTG GAC GTA        820
His Arg Arg Phe Leu Leu Phe Arg Arg Ala Phe Lys Gln Leu Asp Val
    120                 125                 130

GAA GCA GCT CTG ACC AAA GCC CTT GGG GAA GTG GAC ATT CTT CTG ACC        868
Glu Ala Ala Leu Thr Lys Ala Leu Gly Glu Val Asp Ile Leu Leu Thr
135                 140                 145                 150

TGG ATG CAG AAA TTC TAC AAG CTC TGAATGTCTA GACCAGGACC TCCCTCCCCC       922
Trp Met Gln Lys Phe Tyr Lys Leu
                155

TGGCACTGGT TTGTTCCCTG TGTCATTTCA AACAGTCTCC CTTCCTATGC TGTTCACTGG      982

ACACTTCACG CCCTTGGCCA TGGGTCCCAT TCTTGGCCCA GGATTATTGT CAAAGAAGTC     1042

ATTCTTTAAG CAGCGCCAGT GACAGTCAGG GAAGGTGCCT CTGGATGCTG TGAAGAGTCT     1102

ACAGAGAAGA TTCTTGTATT TATTACAACT CTATTTAATT AATGTCAGTA TTTCAACTGA     1162

AGTTCTATTT ATTTGTGAGA CTGTAAGTTA CATGAAGGCA GCAGAATATT GTGCCCCATG     1222

CTTCTTTACC CCTCACAATC CTTGCCACAG TGTGGGGCAG TGGATGGGTG CTTAGTAAGT     1282

ACTTAATAAA CTGTGGTGCT TTTTTTGGCC TGTCTTTGGA TTGTTAAAAA ACAGAGAGGG     1342

ATGCTTGGAT GTAAAACTGA ACTTCAGAGC ATGAAAATCA CACTGTCTGC TGATATCTGC     1402

AGGGACAGAG CATTGGGGTG GGGGTAAGGT GCATCTGTTT GAAAAGTAAA CGATAAAATG     1462

TGGATTAAAG TGCCCAGCAC AAAGCAGATC CTCAATAAAC ATTTCATTTC CCACCCACAC     1522

TCGCCAGCTC ACCCCATCAT CCCTTTCCCT TGGTGCCCTC CTTTTTTTTT TATCCTAGTC     1582

ATTCTTCCCT AATCTTCCAC TTGAGTGTCA AGCTGACCTT GCTGATGGTG ACATTGCACC     1642

TGGATGTACT ATCCAATCTG TGATGACATT CCCTGCTAAT AAAAGACAAC ATAACTCA       1700
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 206 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Asn Phe Gln Gln Arg Leu Gln Ser Leu Trp Thr Leu Ala Arg Pro
-48         -45                 -40                 -35

Phe Cys Pro Pro Leu Leu Ala Thr Ala Ser Gln Met Gln Met Val Val
        -30                 -25                 -20

Leu Pro Cys Leu Gly Phe Thr Leu Leu Leu Trp Ser Gln Val Ser Gly
    -15                 -10                 -5

Ala Gln Gly Gln Glu Phe His Phe Gly Pro Cys Gln Val Lys Gly Val
  1               5                  10                  15

Val Pro Gln Lys Leu Trp Glu Ala Phe Trp Ala Val Lys Asp Thr Met
            20                  25                  30

Gln Ala Gln Asp Asn Ile Thr Ser Ala Arg Leu Leu Gln Gln Glu Val
        35                  40                  45

Leu Gln Asn Val Ser Asp Ala Glu Ser Cys Tyr Leu Val His Thr Leu
    50                  55                  60

Leu Glu Phe Tyr Leu Lys Thr Val Phe Lys Asn Tyr His Asn Arg Thr
 65                  70                  75                  80

Val Glu Val Arg Thr Leu Lys Ser Phe Ser Thr Leu Ala Asn Asn Phe
                85                  90                  95

Val Leu Ile Val Ser Gln Leu Gln Pro Ser Gln Glu Asn Glu Met Phe
            100                 105                 110

Ser Ile Arg Asp Ser Ala His Arg Arg Phe Leu Leu Phe Arg Arg Ala
        115                 120                 125

Phe Lys Gln Leu Asp Val Glu Ala Ala Leu Thr Lys Ala Leu Gly Glu
    130                 135                 140

Val Asp Ile Leu Leu Thr Trp Met Gln Lys Phe Tyr Lys Leu
145                 150                 155
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 178 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Met His Ser Ser Ala Leu Leu Cys Cys Leu Val Leu Leu Thr Gly Val
1               5                   10                  15

Arg Ala Ser Pro Gly Gln Gly Thr Gln Ser Glu Asn Ser Cys Thr His
            20                  25                  30

Phe Pro Gly Asn Leu Pro Asn Met Leu Arg Asp Leu Arg Asp Ala Phe
        35                  40                  45

Ser Arg Val Lys Thr Phe Phe Gln Met Lys Asp Gln Leu Asp Asn Leu
    50                  55                  60

Leu Leu Lys Glu Ser Leu Leu Glu Asp Phe Lys Gly Tyr Leu Gly Cys
65                  70                  75                  80

Gln Ala Leu Ser Glu Met Ile Gln Phe Tyr Leu Glu Glu Val Met Pro
                85                  90                  95

Gln Ala Glu Asn Gln Asp Pro Asp Ile Lys Ala His Val Asn Ser Leu
            100                 105                 110

Gly Glu Asn Leu Lys Thr Leu Arg Leu Arg Leu Arg Arg Cys His Arg
        115                 120                 125

Phe Leu Pro Cys Glu Asn Lys Ser Lys Ala Val Glu Gln Val Lys Asn
    130                 135                 140

Ala Phe Asn Lys Leu Gln Glu Lys Gly Ile Tyr Lys Ala Met Ser Glu
145                 150                 155                 160

Phe Asp Ile Phe Ile Asn Tyr Ile Glu Ala Tyr Met Thr Met Lys Ile
                165                 170                 175

Arg Asn
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 171 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Leu Val Asn Phe Ile Leu Arg Cys Gly Leu Leu Leu Val Thr Leu
1               5                   10                  15

Ser Leu Ala Ile Ala Lys His Lys Gln Ser Ser Phe Thr Lys Ser Cys
            20                  25                  30

Tyr Pro Arg Gly Thr Leu Ser Gln Ala Val Asp Ala Leu Tyr Ile Lys
        35                  40                  45

Ala Ala Trp Leu Lys Ala Thr Ile Pro Glu Asp Arg Ile Lys Asn Ile
    50                  55                  60

Arg Leu Leu Lys Lys Lys Thr Lys Lys Gln Phe Met Lys Asn Cys Gln
65                  70                  75                  80

Phe Gln Glu Gln Leu Leu Ser Phe Phe Asn Glu Asp Val Phe Gly Gln
                85                  90                  95

Leu Gln Leu Gln Gly Cys Lys Lys Ile Arg Phe Val Glu Asp Phe His
            100                 105                 110

Thr Leu Arg Gln Lys Leu Ser His Cys Ile Ser Cys Ala Ser Ser Ala
        115                 120                 125

Arg Glu Met Lys Ser Ile Thr Arg Met Lys Arg Ile Phe Tyr Arg Ile
    130                 135                 140

Gly Asn Lys Gly Ile Tyr Lys Ala Ile Ser Glu Leu Asp Ile Leu Leu
145                 150                 155                 160

Ser Trp Ile Lys Lys Leu Leu Glu Ser Ser Gln
                165                 170
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1197 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
- (A) NAME/KEY: CDS
- (B) LOCATION: 256..798

(ix) FEATURE:
- (A) NAME/KEY: mat_peptide
- (B) LOCATION: 325..798

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
TATCAGGCGA GACGTTTGTT TGGTCCTATG GTAGTCTCAA AGCAGAGTGC ATGGTATCAT      60

GTGGCAGGAA GGCACGGACA AAGCTGAGCT GAAGTGGTTT TCACAAAGTA CCCACTCCAA     120

TGCATACATT CATGGGTTTG TTTAAGAGGG CAGAGATCTG GTAACAGATC TGCGTGTAAG     180

TTCCGAGTCA GAATTTGACT TCAGGGTAAA GCCTTCCTTT CTTCAGCAGG AGCACTGGCC     240

CTTTCTTCAA CACAG ATG AGT TGG GGA CTA CAG ATT CTC CCC TGC CTG AGC      291
                 Met Ser Trp Gly Leu Gln Ile Leu Pro Cys Leu Ser
                 -23         -20                 -15

CTA ATC CTT CTT CTT TGG AAC CAA GTG CCA GGG CTT GAG GGT CAA GAG       339
Leu Ile Leu Leu Leu Trp Asn Gln Val Pro Gly Leu Glu Gly Gln Glu
    -10                 -5                   1               5

TTC CGA TCG GGG TCT TGC CAA GTG ACA GGG GTG GTT CTC CCA GAA CTG       387
Phe Arg Ser Gly Ser Cys Gln Val Thr Gly Val Val Leu Pro Glu Leu
                10                  15                  20

TGG GAG GCC TTC TGG ACT GTG AAG AAC ACT GTG CAA ACT CAG GAT GAC       435
Trp Glu Ala Phe Trp Thr Val Lys Asn Thr Val Gln Thr Gln Asp Asp
            25                  30                  35

ATC ACA AGC ATC CGG CTG TTG AAG CCG CAG GTT CTG CGG AAT GTC TCG       483
Ile Thr Ser Ile Arg Leu Leu Lys Pro Gln Val Leu Arg Asn Val Ser
        40                  45                  50

GGT GCT GAG AGC TGT TAC CTT GCC CAC AGC CTG CTG AAG TTC TAC TTG       531
Gly Ala Glu Ser Cys Tyr Leu Ala His Ser Leu Leu Lys Phe Tyr Leu
    55                  60                  65

AAC ACT GTT TTC AAG AAC TAC CAC AGC AAA ATA GCC AAA TTC AAG GTC       579
Asn Thr Val Phe Lys Asn Tyr His Ser Lys Ile Ala Lys Phe Lys Val
70                  75                  80                  85

TTG AGG TCA TTC TCC ACT CTG GCC AAC AAC TTC ATA GTC ATC ATG TCA       627
Leu Arg Ser Phe Ser Thr Leu Ala Asn Asn Phe Ile Val Ile Met Ser
                90                  95                  100

CAA CTA CAG CCC AGT AAG GAC AAT TCC ATG CTT CCC ATT AGT GAG AGT       675
Gln Leu Gln Pro Ser Lys Asp Asn Ser Met Leu Pro Ile Ser Glu Ser
            105                 110                 115

GCA CAC CAG CGG TTT TTG CTG TTC CGC AGA GCA TTC AAA CAG TTG GAT       723
Ala His Gln Arg Phe Leu Leu Phe Arg Arg Ala Phe Lys Gln Leu Asp
        120                 125                 130

ACA GAA GTC GCT TTG GTG AAA GCC TTT GGG GAA GTG GAC ATT CTC CTG       771
Thr Glu Val Ala Leu Val Lys Ala Phe Gly Glu Val Asp Ile Leu Leu
    135                 140                 145
```

```
ACC TGG ATG CAG AAA TTC TAC CAT CTC TGACTGCTGA TTGGATAACT          818
Thr Trp Met Gln Lys Phe Tyr His Leu
150                 155

TCCTCCTTTG CTCTCCATGC CATTTCAAGG CATTGTGTAC ATCCCTGCTG TCCTCAAGGC   878

ACTTCAGACC CTTGGCCATG GACCCCGTTG TTGGCTCAGG CTTTTCCTCA GACCTCACTC   938

TTCAGTCCAA ATGACAGCCA TAGATGGCAC CTTTGGATGC TCCGACTGAC CCACAAAGTA   998

GATTTGCATA TTTATTACAG CCCTATTAAA TTATTGTCAC CTTCCCTGGA AACCGTATTT  1058

ATTTGTGAGA CCAGAAGTTC CATGAAAGCA TCAGAATTTA GTGCCCCATG CCTCCTCCTC  1118

ACTTCCTGTG ATCTGGCTCA GCATGGGGGC AGTGGATGGT TGCTCAGTAA ATATTTAAAA  1178

TGGAAAAAAA AAAAAAAAA                                                1197
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 181 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Met Ser Trp Gly Leu Gln Ile Leu Pro Cys Leu Ser Leu Ile Leu Leu
-23         -20                 -15                 -10

Leu Trp Asn Gln Val Pro Gly Leu Glu Gly Gln Glu Phe Arg Ser Gly
        -5                  1               5

Ser Cys Gln Val Thr Gly Val Val Leu Pro Glu Leu Trp Glu Ala Phe
 10                  15                  20                  25

Trp Thr Val Lys Asn Thr Val Gln Thr Gln Asp Asp Ile Thr Ser Ile
                30                  35                  40

Arg Leu Leu Lys Pro Gln Val Leu Arg Asn Val Ser Gly Ala Glu Ser
            45                  50                  55

Cys Tyr Leu Ala His Ser Leu Leu Lys Phe Tyr Leu Asn Thr Val Phe
        60                  65                  70

Lys Asn Tyr His Ser Lys Ile Ala Lys Phe Lys Val Leu Arg Ser Phe
    75                  80                  85

Ser Thr Leu Ala Asn Asn Phe Ile Val Ile Met Ser Gln Leu Gln Pro
 90                  95                 100                 105
```

```
Ser Lys Asp Asn Ser Met Leu Pro Ile Ser Glu Ser Ala His Gln Arg
                110                 115                 120

Phe Leu Leu Phe Arg Arg Ala Phe Lys Gln Leu Asp Thr Glu Val Ala
            125                 130                 135

Leu Val Lys Ala Phe Gly Glu Val Asp Ile Leu Leu Thr Trp Met Gln
        140                 145                 150

Lys Phe Tyr His Leu
    155
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 178 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
Met Pro Gly Ser Ala Leu Leu Cys Cys Leu Leu Leu Leu Thr Gly Met
1               5                   10                  15

Arg Ile Ser Arg Gly Gln Tyr Ser Arg Glu Asp Asn Asn Cys Thr His
            20                  25                  30

Phe Pro Val Gly Gln Ser His Met Leu Leu Glu Leu Arg Thr Ala Phe
        35                  40                  45

Ser Gln Val Lys Thr Phe Phe Gln Thr Lys Asp Gln Leu Asp Asn Ile
    50                  55                  60

Leu Leu Thr Asp Ser Leu Met Gln Asp Phe Lys Gly Tyr Leu Gly Cys
65                  70                  75                  80

Gln Ala Leu Ser Glu Met Ile Gln Phe Tyr Leu Val Glu Val Met Pro
                85                  90                  95

Gln Ala Glu Lys His Gly Pro Glu Ile Lys Glu His Leu Asn Ser Leu
            100                 105                 110

Gly Glu Lys Leu Lys Thr Leu Arg Met Arg Leu Arg Arg Cys His Arg
        115                 120                 125

Phe Leu Pro Cys Glu Asn Lys Ser Lys Ala Val Glu Gln Val Lys Ser
    130                 135                 140

Asp Phe Asn Lys Leu Gln Asp Gln Gly Val Tyr Lys Ala Met Asn Glu
145                 150                 155                 160
```

```
Phe Asp Ile Phe Ile Asn Cys Ile Glu Ala Tyr Met Met Ile Lys Met
                165                 170                 175

Lys Ser
```

WHAT IS CLAIMED IS:

1. A substantially pure or recombinant soluble IL-BKW protein.

2. An antigenic protein or peptide fragment of the IL-BKW of claim 1.

3. The IL-BKW of claim 2, which is a full length natural soluble protein from a mammal, including a primate or mouse.

4. The IL-BKW of claim 2, which:
  a) is a soluble IL-BKW lacking the sequence MNFQQRLQSL WTLARPFCPP LLATASQMQM VVLPCLGFTL LLWSQVSG of SEQ ID NO: 2;
  b) is a mature polypeptide of SEQ ID NO: 6; or
  c) is encoded by a nucleic acid of SEQ ID NO: 5.

5. The IL-BKW of claim 2, which is a full length secreted protein which exhibits a post-translational modification pattern distinct from natural IL-BKW.

6. The IL-BKW of claim 2, which exhibits an antagonist immunological activity of IL-10.

7. A fusion protein comprising sequence of a protein or peptide of claim 2, wherein said protein or peptide:
  a) lacks a sequence of MNFQQRLQSL WTLARPFCPP LLATASQMQM VVLPCLGFTL LLWSQVSG of SEQ ID NO: 2;
  b) is a mature polypeptide of SEQ ID NO: 6; or
  c) is encoded by a nucleic acid of SEQ ID NO: 5.

8. A sterile composition comprising a protein or peptide of claim 2.

9. A method of purifying an IL-BKW protein or peptide of claim 6 from other materials in a mixture comprising contacting said mixture to an antibody to said protein, and separating bound IL-BKW from other materials.

10. An isolated or recombinant expression vector encoding a soluble IL-BKW of claim 1.

11. The vector of claim 10, wherein said nucleic acid encodes a secreted sequence of SEQ ID NO: 2 or 6

12. The vector of claim 10, which comprises a sequence of SEQ ID NO: 1 or 5.

13. A kit for detection comprising a positive control which is a substantially pure soluble IL-BKW or fragment of claim 1.

14. A method for detecting in a sample for the presence of an IL-BKW nucleic acid, protein, or antibody, comprising testing said sample with a kit of claim 11.

15. A method of modulating the physiology of a cell comprising contacting said cell with a substantially pure soluble IL-BKW of claim 1.

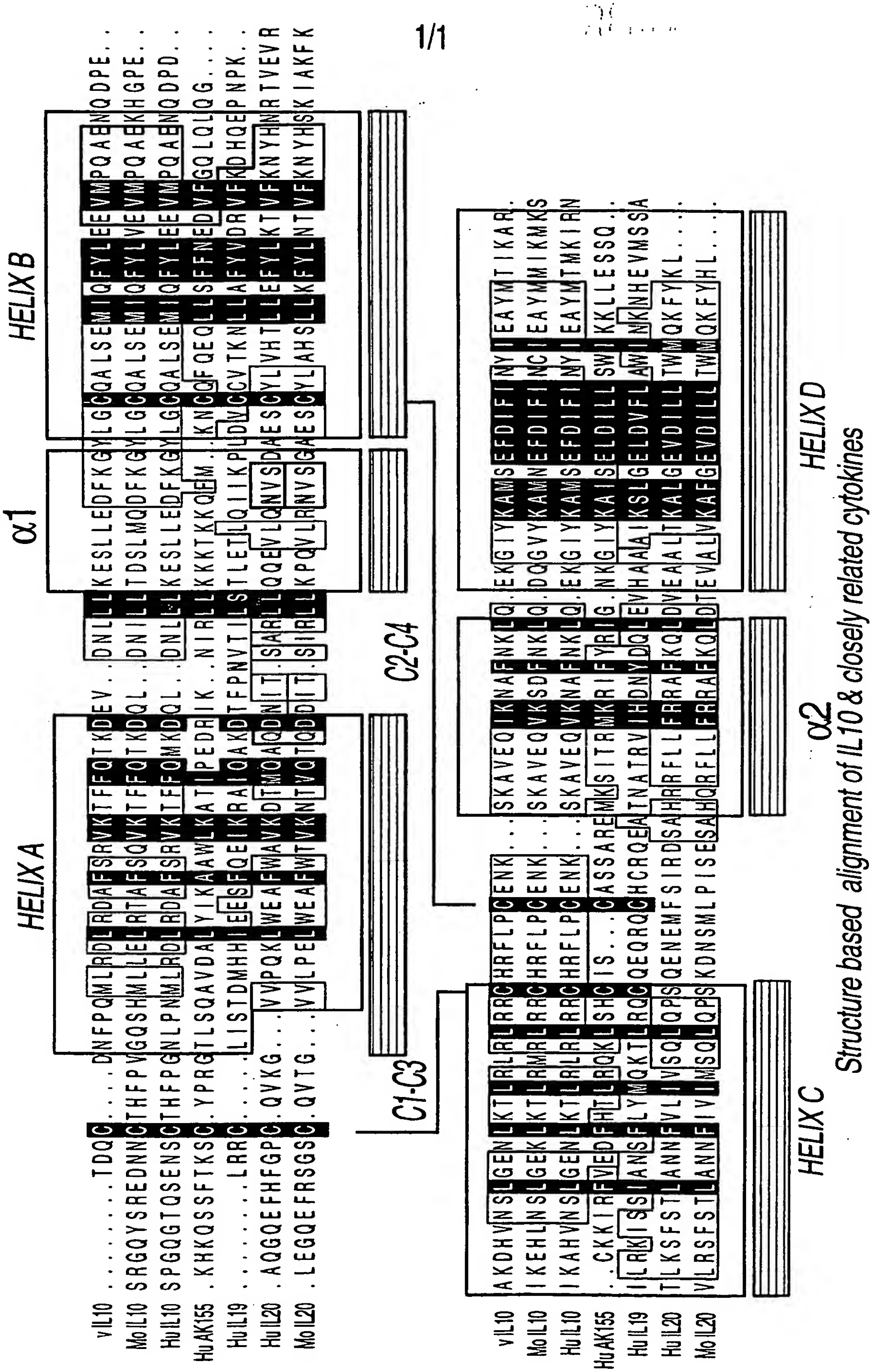
16. The method of claim 15, wherein said cell is a T cell and said modulating of physiology is inactivation of said T cell.

17. The method of claim 15, wherein said cell is in a tissue.

18. A method of making a soluble IL-BKW comprising expressing a vector of claim 10.

19. A cell, tissue, or organ comprising a vector of claim 10.

20. A method of treating a mammal having an abnormal immune response by administering to said mammal an effective dose of a substantially pure soluble IL-BKW.



Structure based alignment of IL10 & closely related cytokines

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/22975

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/19 C07K14/54 C12N15/62 G01N33/50 C12Q1/68 A61K38/19 C12N5/10 A01K67/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K G01N C12Q A61K A01K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JIANG H ET AL: "Substraction hybridization identifies a novel melanoma differentiation associated gene, mda-7, modulated during human melanoma differentiation, growth and progression" ONCOGENE, vol. 11, 1995, pages 2477-2486, XP002064717 cited in the application see page 2484, right-hand column, last paragraph - page 2485; figure 1 ---	1-6, 8-14,18, 19
X	WO 95 11986 A (UNIV COLUMBIA) 4 May 1995 see page 223 - page 228; figure 38 see page 324 - page 329 -----	1-6, 8-14,18, 19

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 May 1998	20.05.98
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Espen, J

INTERNATIONAL SEARCH REPORT	national application No. PCT/US 97/22975

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See continuation-sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-3,5,6,8-10,13,15-20; in part 4,7,11,12,14

   IL-BKW protein, fusion protein comprising a sequence of IL-BKW protein, method of purifying an IL-BKW protein, vector encoding an IL-BKW protein, kit for detection, method for detection, method of modulating the physiology of a cell; cell,tisue, or organ comprising vector encoding an IL-BKW protein

2. Claims: in part: 4,7,1,12,14

   IL-BKW protein lacking the sequence of amino acid residues 1-48 of SEQ ID NO 2, fusion protein comprising said protein, vector encoding said protein, method for detection

INTERNATIONAL SEARCH REPORT

..formation on patent family members

Inter   nal Application No

PCT/US 97/22975

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9511986 A	04-05-1995	US 5643761 A	01-07-1997
		AU 8088394 A	22-05-1995
		EP 0730659 A	11-09-1996
		JP 9507021 T	15-07-1997